Parasites and life history variation in a wild mammal

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Declaration

I have composed this thesis. All analyses presented in this thesis are my own work. The long-term data used in this thesis has been collected by others as part of the ongoing St Kilda Soay sheep project. The antibody assays described in chapter 6 were conducted by Kathryn Watt. I have participated in the collection of data and have full understanding of the methods used to collect all of the data used in this thesis. I composed all of the chapters in this thesis, incorporating comments from co-authors and supervisors. This work has not been submitted for any other professional degree or qualification.

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Abstract

The purpose of this thesis is to investigate associations between parasite infection and host life-history variation in the wild Soay sheep population of the islands of St Kilda, NW Scotland. Studying host-parasite interactions in wild animal populations is of interest because of the importance of heterogeneity in resource availability, genetics, and environmental conditions in determining resistance to parasites, with implications for human populations and wildlife conservation and management. However, very few studies are able to investigate these associations in a longitudinal manner, which is essential in order to understand how infection is associated with life-history variation across ages and environmental conditions. In this thesis, I investigate associations between parasite resistance and ageing and the importance of maternal effects on offspring parasite resistance. I also establish the shape of natural selection on parasite resistance, and associations between measures of parasite burden and antibody responses. The principle findings of the analyses presented in this thesis are:

i) Adult sheep of both sexes show a decline in parasite resistance in old age which is consistent with senescence. Furthermore, the rate of decline in parasite resistance with age is accelerated in individuals that have experienced more stressful environmental conditions over their lifespan.

ii) Aspects of maternal phenotype and lamb early life performance are significantly associated with parasite resistance in lambs. Some of these effects persist into adult life and may even affect late-life changes in parasite resistance with age.

iii) Analysis of ageing in five female reproductive traits shows that the contributions of individual senescence, terminal effects, and selective disappearance vary across traits, and that therefore multiple traits should be studied in order to understand ageing more fully. Most strikingly, there was no evidence for significant senescence in the probability of producing twins.

iv) The first estimate of the strength of natural selection on parasite resistance in a longitudinally-monitored population provided evidence for positive selection on parasite resistance in lambs but not adults. Selection in lambs also varied across environmental conditions, being stronger in years of more favourable conditions.

v) Analysis of associations between estimates of parasite burden and antibody responses showed that an estimate of parasite burden was not correlated with either a general or parasite-specific antibody response. However, antibody responses were positively correlated, and there was some evidence for a genetic correlation between the two in lambs but not adults.

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Chapter 1

General Introduction

Parasitism is one of the most successful life-history strategies that have ever evolved, and indeed the majority of living organisms are parasites (Hudson *et al*., 2002). Parasites have a major impact on human populations, with direct effects in terms of mortality and morbidity, and indirect effects such as the economic costs of parasites of crops and livestock. For instance, it has been estimated that 2 billion people worldwide are infected with parasitic helminths (Colley *et al*., 2001), and in the UK sheep industry alone helminth infections are estimated to cause productivity losses worth £84 million and the spending of another £70 million on anthelmintic drugs (Nieuwhof & Bishop, 2005; Jackson *et al*., 2009a). Parasites and emerging diseases also have a large impact on wild animal populations, with important consequences for wildlife management and conservation (Cleaveland *et al*., 2002; Pedersen *et al*., 2007; McCallum *et al*., 2009). Knowledge of the impacts of parasites and host-parasite interactions is far less detailed in wildlife populations than in humans or domesticated animals, and so studies of the causes and consequences of variation in parasite infections in natural populations are vital. The results of such studies are likely to have important implications for human and wildlife populations, and study in natural settings will complement experimental work to provide a clearer picture of host-parasite interactions in nature.

The aim of this thesis is to investigate the associations between macroparasite infection and variation in host life-history traits in the free-living Soay sheep (*Ovis aries*) population on the islands of St Kilda, NW Scotland. The population has been studied for over twenty years, and has collected detailed longitudinal data on life-history traits, morphometric measurements,

parasite infections, and environmental conditions; a detailed pedigree has also been constructed (Clutton-Brock & Pemberton, 2004). The parasite species infecting individuals in the population have been well-characterized, the most prevalent of which are the strongyle nematodes (Wilson *et al*., 2004), a group of gastrointestinal macroparasites which are responsible for the economic losses in domesticated sheep described above. An inverse estimate of individual parasite resistance is gained from faecal egg counts (FEC), the number of parasite eggs found per gram of sheep faeces, which is a commonly used estimate of parasite resistance in domesticated populations (Sayers & Sweeney, 2005). FEC is repeatable among individuals and has significant additive genetic variance in this population (Coltman *et al*., 2001a; Beraldi *et al*., 2007), and its use as a measure of parasite resistance is discussed in section 1.5.5 and in chapter 5.

Associations between FEC and survival, morphometrics, and seasonality have been relatively well-studied in the Soay sheep population. However, several gaps remain in our knowledge of the associations between parasites and host life-history variation, and the objective of this thesis is to attempt to fill some of these gaps. Associations between ageing and parasite resistance are poorly understood, including the factors associated with early development of parasite resistance and the effects of senescence on parasite resistance in later life. The effect of senescence on reproductive performance has yet to be studied, and nothing is known about the impact of parasites on senescence in such traits in this or any other natural population. Natural selection on morphometric traits has been investigated in this population, but there are no estimates of the strength of natural selection on parasite resistance in this or in any other wild population. Finally, it is unknown how apparent resistance to parasites is related to investment in immune function in this population. All of this is investigated in a food-limited population experiencing heterogeneous environmental conditions, the importance of which will also be determined.

This introductory chapter reviews the literature that has inspired the lines of investigation in this thesis. Firstly, I discuss the current understanding of the distribution of macroparasites in natural populations, and some of the major sources of the observed variation in parasite resistance among hosts, with particular reference to the Soay sheep of St Kilda. I then describe work on aspects of the host-parasite relationship in natural settings, and in particular the effects of parasites on hosts in terms of population regulation and effects on individuals. I briefly discuss the emerging field of wild immunology, and how measures of immune function can inform the observed interactions between hosts and parasites. Finally, I introduce the study system and the methods of data collection.

1.1 The distribution of macroparasites

Multicellular parasites such as helminths and arthropods, often referred to as macroparasites, are rarely randomly distributed amongst hosts. Even within host species and populations, the usual pattern is one of aggregation, with the majority of hosts harbouring few parasites, and a few hosts harbouring the majority of parasites in the population (Anderson & May, 1978; Shaw & Dobson, 1995). Parasite burdens are therefore highly variable, with variation in factors both extrinsic and intrinsic to the host contributing to individual parasite counts (Wilson *et al*., 2002). Extrinsic effects are largely environmental, and related to exposure to parasites, while intrinsic factors refer to heterogeneity in parasite burden associated with behavioural, hormonal, genetic, and condition-based differences in resistance between individuals. This right-skewed distribution of parasite counts is referred to as overdispersion, and is characterized by a variance to mean ratio of greater than one (Crawley, 2007). Such distributions present problems when performing statistical analysis of parasite count data using traditional parametric methods, a problem which

has conventionally been accounted for by log-transforming parasite count data so that it approximates a normal distribution. However, the use of generalized linear models (GLMs) has been strongly advocated (e.g. Wilson & Grenfell, 1997), with analysis using negative binomial error distributions consistently shown to out-perform data analysed using parametric tests on log-transformed data (e.g. Wilson *et al*., 1996; Shaw *et al*., 1998). The negative binomial distribution does not always best describe parasite count data (e.g. Stear *et al*., 2006), and so other techniques have been explored (e.g. Elston *et al*., 2001; ver Hoef *et al*., 2007). However, the negative binomial distribution within the GLM framework and its extensions is now the most frequently used approach for analyzing parasite count data in natural populations (Paterson & Lello, 2003). The statistical issues associated with parasite count data are discussed more specifically in chapters 2 and 3.

1.2 Causes of variation in parasite burden

One of the central aims of epidemiology, ecology, and evolutionary biology is explaining the sources of variation in infection intensities within and between individuals and populations, and determining the aspects of host phenotype which are associated with parasite resistance (discussed extensively in Wilson *et al*., 2002). Below, I will briefly outline some of the most important sources of variation and highlight their relevance to the Soay sheep of St Kilda.

1.2.1 Spatial and temporal variation

The majority of natural habitats are highly heterogeneous, with spatial and temporal variation in macro- and micro-climate, levels of competition, nutrients, and shelter. Animals are usually not

static, exhibiting some form of territorial behaviour or home-ranging, and so only sample a small proportion of the habitat available to the whole population. These factors mean that individual exposure to parasites is likely to differ across spatial and temporal scales. One of the most important sources of variation is host population density, with the expectation that the probability of infection with a directly transmitted parasite should increase with the rate of contact with conspecifics, and therefore that host populations at higher density should experience higher levels of parasitism (Arneberg *et al*., 1998; Morand & Poulin, 1998; Telfer *et al*., 2007; Lindsey *et al*., 2009; Radwan *et al*., 2010).

As well as changing between years, environmental conditions may be highly variable within years, and many studies have shown intra-annual variation in parasite species richness and infection intensity (Telfer *et al*., 2007; Simões *et al*., 2010; Turner *et al*., 2010). The mechanisms determining seasonal changes in parasitism include climatic variation and host and parasite phenology (Hawley & Altizer, 2011), and have been particularly well-studied in domestic sheep, which, although managed, are subject to changes in environmental conditions out on the pasture. The greatest seasonal change in nematode parasite burdens in domestic sheep is the peri-parturient rise (PPR), an increase in infection intensity which occurs around the lambing period (Kassai, 1999), and which is explained by both host and parasite biology. Females carrying lambs are thought to be immunosuppressed in order to avoid rejection of the lamb (Barger 1993; Huntley *et al*., 2004), and they are also of lower nutritional status due to the demands of pregnancy and lactation; these factors result in females being less resistant to helminths (Houdijk, 2008). Meanwhile, nematode parasite larvae arrest development either on the pasture or in the host gut mucosa in response to changing environmental conditions (Michel *et al*., 1974; Langrova *et al*., 2008). The reactivation of these arrested larvae results in large increases in infective stages on pasture and fecundity of worms already infecting sheep, and so exposure and

FEC both increase around the early spring (Uriate *et al*., 2003). This pattern is observed in St Kilda Soay sheep, with an additional peak of infection later in the year when worms infecting lambs mature and begin shedding eggs onto pasture (Gulland & Fox, 1992; Wilson *et al*., 2004).

The above factors result in variation in exposure in time, and are large-scale effects that influence the whole population, but it is also known that heterogeneity in microhabitats can affect exposure within the population, and cause variation in the prevalence or intensity of infection across spatial scales (e.g. Byers *et al*., 2008; Osnas *et al*., 2009). For instance, if development of nematode larvae on pasture requires moisture, it is expected that transmission of these parasites in moister areas of a habitat should be higher (O'Connor *et al*., 2006). Variation in exposure across temporal and spatial scales can therefore result in individuals with different infection histories and account for some of the variation seen within and between populations in parasite species richness, prevalence, and infection intensities.

1.2.2 Host sex

One of the host traits most strongly linked to variation in prevalence and intensity of infection is host sex, with intersexual differences in behaviour, life history strategy, and investment in resistance all implicated, and males usually experiencing higher parasitism (Zuk & McKean, 1996). A simple explanation for this pattern is that males are larger, and therefore represent larger targets for parasites (Arneberg, 2002; Harrison *et al*., 2010). Another explanation is that since investment in immunity is costly, resource allocation strategies may differ between the sexes, with males investing higher levels in, for instance, reproductive effort, and females investing more in parasite resistance. The endocrine system is known to interact with the

immune system, and it has been shown that a variety of hormones are associated with immunosuppression costs (Klein, 2004). Testosterone in particular has been has been implicated in changes in behaviour and physiology which increase susceptibility to infection and reduce parasite resistance (e.g. Seivwright *et al*., 2005; Grear *et al*., 2009).

There is a male sex bias in infection with gastrointestinal nematodes in St Kilda Soay sheep, with males exhibiting higher prevalence and intensity of infection (Gulland & Fox, 1992). There is pronounced adult size dimorphism, with females around two-thirds the body weight of males (Clutton-Brock *et al*., 1996; Milner *et al*., 1999a), but dimorphism in lambs is relatively low, with males only 4% heavier on average at birth and not significantly heavier at four months of age (Clutton-Brock *et al*., 2004a). Despite this, at just ten weeks of age, FEC is on average 60% higher in males than females, and by six months of age, male FEC is double that of females (Wilson *et al*., 2004). Hence, body size cannot fully explain this male bias, and an experimental study on a mainland domesticated population of Soay sheep has implicated differences in allocation to immunity (Gulland, 1991). Naïve lambs of both sexes received a dose of 1400 larvae of the gastrointestinal nematode *Teladorsagia circumcincta*; after 28 days, sheep were euthanized and it was found that a higher proportion of larvae survived to adulthood in males than in females (Gulland, 1991). Thus, as in many systems, it seems that male Soay sheep have lower resistance, and therefore may experience a greater cost of parasite infection than females.

1.2.3 Host age and senescence

In natural vertebrate populations, the typical pattern of age-specific change in phenotypic traits is a bell-shaped curve, with low performance in early life, a peak in middle age, and a decline

during old age (for recent examples see Bouwhuis *et al*., 2009; Nussey *et al*. 2009a; Lecomte *et al*., 2010; Sharp *et al*., 2010). Studies of parasite infection intensity and parasite resistance have tended to focus on the first half of this curve and the development of parasite resistance during the early part of life, as well as the differences between juveniles and mature adults. A common finding is for juveniles to show higher prevalence or infection intensities than adults (e.g. Isomursu *et al*., 2006; Chylinski *et al*., 2009), and this is the pattern observed in St Kilda Soay sheep (Craig *et al*., 2008). This pattern may be explained by differences in behaviour and exposure between juveniles and adults or development of resistance in adults (Wilson *et al*., 2004). However, changes in parasite resistance across adult ages are rarely studied.

Evolutionary theory predicts that life-history traits should show declines in late life (senescence), since natural selection weakens with age and so there is weaker selection against deleterious mutations with late-life effects (Medawar, 1952; Williams, 1957; Hamilton, 1966; Kirkwood, 1977). Hence, theory predicts that individuals should become more susceptible to infection and have lower immune function in old age, a phenomenon which is well-established in the medical literature (e.g. Pawalec, 1997; Gavazzi & Krauze 2002; Gruver *et al*., 2007), but studies of senescence in parasite resistance or immune function in natural populations are rare. This may be due to several reasons: the assumption that ageing does not occur because animals die due to predation, disease or starvation before they show senescence (Rose, 1991; Hayflick, 2000); the difficulty of collecting sufficient data from the oldest age classes; or the difficulty of longitudinal sampling of individuals in order to make accurate observations about the way in which traits change with age (Nussey *et al*., 2008). The issues associated with testing for within-individual ageing are discussed in detail in chapters 2 and 4.

Most studies of parasite resistance and ageing tend be cross-sectional studies on a global measure of immune function indicative of the overall strength of the immune response. Findings have been mixed, with some studies showing that measures of immune function decline in old age (e.g. Cichon *et al*., 2003; Lozano & Lank, 2003; Saino *et al*., 2003; Haussmann *et al*., 2005; Palacios *et al*., 2007) , and some finding an increase in performance or stasis (e.g. Palacios *et al*., 2007; Lecomte *et al*., 2010). It is difficult to make predictions based on these studies, since they examine population- rather than individual-level trends and use a variety of measures of immune function, some of which have been heavily criticised (e.g. Adamo, 2004; Bradley & Jackson, 2008). In section 1.4 I will discuss some of the issues to be considered when studying immune responses in natural populations.

1.2.4 Host genetics and behaviour

The phrase 'genetically resistant' is often used to explain why hosts differ in their susceptibility to parasite infection, and indeed it has been shown that certain genetic loci, strains, or heterozygosity levels are associated with enhanced parasite resistance (e.g. Duncan & Little, 2007; Auld *et al*., 2010; Radwan *et al*., 2010). It is, however, difficult to gain estimates of additive genetic variance and the potential for evolution of such traits in natural populations, since it requires detailed sampling of natural variation in parasite resistance and knowledge of the relatedness of individuals. In some human populations, this data has been collected, and it has been shown that measures of susceptibility and resistance may have a strong additive genetic basis (e.g. Quinnell, 2003; Cuenco *et al*., 2009). In domestic sheep populations, resistance to gastrointestinal nematodes is estimated from faecal egg counts (FEC), or the number of parasite eggs per gram of faeces. Quantitative genetic analysis of FEC and antibody responses have

shown that both have a significant additive genetic component (Bishop *et al*., 1996; Stear *et al*., 1996; Stear *et al*., 1999; Strain *et al*., 2002; Stear *et al*., 2009), and FEC is used as a target trait in selective breeding for enhanced parasite resistance in domesticated populations (Sayers & Sweeney, 2005). Longitudinal blood and faecal sampling, and a detailed pedigree, have allowed similar studies on the genetics of parasite resistance in Soay sheep to be examined.

FEC in Soay sheep has a significant additive genetic basis (Coltman *et al*., 2001a; Beraldi *et al*., 2007). It has also been shown that certain MHC alleles are associated with high or low FEC (Paterson *et al*., 1998), that less heterozygous sheep have higher FEC (Coltman *et al*., 1999a), and that certain genetic loci are significantly associated with FEC (Coltman *et al*., 2001b; Beraldi *et al*., 2007). Recent studies have also found negative genetic correlations between FEC and body size, suggesting that genetically resistant individuals are larger and are likely to have higher fitness (Coltman *et al*., 2001a; Robinson *et al*., 2009). Genetic differences do not only influence immunity or resistance to parasites, but also individual behaviour. A study of grazing behaviour in this population showed that females with lambs avoided parasitized but more nutritious areas of forage more than barren females, suggesting that the response to the trade-off between nutritional value of forage and likelihood of parasite infection changes with host state (Hutchings *et al*., 2002). These observations suggest that FEC may be used as an estimate of individual parasite resistance (discussed further in section 1.5.5 and chapter 5).

1.2.5 Maternal effects

Associations between maternal phenotype and aspects of offspring phenotype not due to genetics or heritable variation (maternal effects) may determine offspring fitness and impact evolutionary

dynamics in natural populations (Mousseau & Fox, 1998; Marshall & Uller, 2007; Rasanen & Kruuk, 2007). Maternal effects including maternal infection status, circulating antibody levels, and condition, may influence offspring immune function and parasite resistance in early life (reviewed by Grindstaff *et al*., 2003); such effects may even persist into adult life (Reid *et al*. 2006). For instance, in a study of pied flycatchers (*Ficedula hypoleuca*), it was found that exposing mothers to bacterial antigen before egg laying resulted in increased offspring antibody production, which was interpreted as mothers preparing offspring for a high probability of infection when they hatched (Grindstaff *et al*., 2006). However, a study of great tits (*Parus major*) reported that natural variation in maternal antibody levels did not correlate with nestling size, antibody production, or tolerance to parasites, and concluded that factors other than antibodies were the most important determinant of early parasite resistance (Tschirren *et al*., 2009). Similarly, a laboratory study of Japanese quail, which injected antibodies into egg yolk sacs, found that there was no effect of antibody supplementation on nestling antibody levels, and that adult antibody levels were only correlated with levels as a juvenile, indicating that post-hatching priming of immunity was more important than pre-hatching effects (Addison *et al*., 2010).

As well as maternal infection status or antibody production, it is thought that maternal condition is of great importance, since antibody production is potentially energetically expensive. A study on Ural owls (*Strix uralensis*) showed that supplementary feeding of mothers increased maternal condition and antibody production, as well as that of their offspring, suggesting that antibody provisioning by mothers is a passive process that is dependent on maternal condition (Karell *et al*., 2008). Although they show different and sometimes contrasting results, the above studies have one thing in common: they are all studies of birds, which can be reliably caught at nest boxes and are easy to manipulate experimentally. Given their particularly high levels of maternal

investment, maternal effects are likely to be equally or even more important in mammals. However, maternal effects on parasite resistance in wild mammals have rarely been studied (but see Kristan, 2004), since they are difficult to reliably capture and re-capture in order to perform the necessary assays.

1.3 The impact of parasites on natural populations

In the preceding section, I discussed sources of heterogeneity in levels of parasite burden and parasite resistance, with a particular emphasis on studies of natural populations. However, in order to understand the dynamics of host-parasite systems across individual life histories and evolutionary timescales, it is essential to understand how parasites affect host fitness, behaviour and resource allocation strategies. Theoretical studies over 30 years ago revealed the potential for parasites to regulate host population dynamics (Anderson & May, 1978; May & Anderson, 1978), and yet there are very few field studies showing that parasites can do so (Tompkins *et al*., 2002). Hudson *et al*. (1998) showed that population crashes of red grouse (*Lagopus lagopus scoticus*) occurred in control populations, but not in populations treated with anthelmintic drugs, suggesting that mortality in population crashes was the result of infection with the gastrointestinal nematode *Trichostrongylus tenuis*. A recent experimental study on two species of free-living mice (white-footed mice *Peromyscus leucopus* and deer mice *P. maniculatus*) studied the simultaneous effects of food supplementation and parasite removal on host mortality and seasonal population crashes (Pedersen & Grieves, 2008). Populations that were either treated with anthelmintic or given additional food showed population crashes of reduced magnitude compared to a control population with natural food levels and parasites present; however, crashes were absent in the population that received anthelmintic treatment and food

supplementation (Pedersen & Grieves, 2008). This suggests that heterogeneity in resource acquisition and availability may be crucial in determining the effects of parasites on hosts, an observation which is particularly pertinent to understanding host-parasite interactions in resource-limited natural populations.

Many experimental and observational studies have shown that parasites can reduce the survival prospects of individual hosts (e.g. van Oosterhout *et al*., 2007; Shuepbach & Baur, 2008; Devevey & Christe, 2009), but they do not consider the fact that parasite resistance may be as costly as parasite infections (Colditz, 2008). The costs of resistance and infection can only be studied together by measuring the full range of natural variation in parasite infection intensity and its association with host fitness. A study of blue tits has shown a non-linear association between infection intensity with the blood parasite *Haemoproteus majoris* and host survival, with birds harbouring moderate parasite infection intensities showing higher survival than those with lower or higher burdens (Stjernman *et al*., 2008), suggesting that hosts that invest some but not all of their resources into parasite resistance have the highest survival (Viney *et al*., 2005). In other cases, parasites may not have a direct effect on host mortality, but they may have important effects on other traits such as body condition or reproductive performance that may negatively influence host fitness. It has been shown that gastrointestinal nematodes can have adverse effects on wild ungulates, with reindeer (*Rangifer tarandus*) treated with anthelmintics showing heavier carcass weight and back fat depth at culling, which was associated with a higher probability of pregnancy (Stien *et al*., 2002). Similarly, an experimental study on wild hares found that anthelmintic-treated females were in better condition upon culling, and were more fecund than untreated females (Newey & Thirgood, 2004).

To date, the effects of parasites on the fitness of Soay sheep have been examined in three experimental studies, all of which analysed effects of anthelmintic treatment on over winter survival. In the first, anthelmintic treatment was administered to 52 sheep in the August of 1988, and was shown to have no effect on survival to the spring of 1989, with 44/52 sheep dying, compared with 34/40 control animals; however, it was shown that treated lambs and female two-year olds survived for longer into the winter (Gulland, 1992). In a second, similar, experiment over the winter of 1991/92, treated female lambs and male yearlings had a significantly higher probability of survival than untreated controls, although treatment had no effect in female yearlings (Gulland *et al*., 1993). Finally, a recent study examined factors influencing the survival of young adults (two-year-olds), and found that anthelmintic treatment had no effect on survival (Craig *et al*., 2009). The results of these studies show that effects on survival may differ across environmental conditions, and across years, although all three of these were winters of exceptionally high mortality. However, although they establish causal effects of parasites on host fitness, they do not assess the associated cost of immunity, as detailed above. No study has tested for complex associations between parasite resistance and fitness in Soay sheep, but it has been shown that FEC is negatively associated with overwinter survival in a linear fashion (Coltman *et al*., 1999a), and it has been speculated that parasite infection may be a factor underlying positive selection on body weight (Milner *et al*., 1999a).

1.4 Wild immunology

At the interface of host and parasite is the host immune system, and it is only by understanding how host immunity regulates and is regulated by parasites that observations on phenotypic traits such as parasite burden, host resistance, and tolerance can be understood (Bradley & Jackson,

2008; Raberg *et al*., 2009; Graham *et al*., 2011). Although the epidemiology, population dynamic consequences, and causes of variation in wildlife diseases have been and are continuing to be relatively well-studied (Grenfell *et al*., 2002), immune responses in natural vertebrate populations are extremely poorly characterised. Studies of immune responses in wild animals could aid understanding of human immunology, since these are more similar to the majority of human populations where helminth infections are endemic than are studies of genetically uniform laboratory animals that are infected with only one parasite and fed *ad libitum* (Pedersen & Babayan, 2011). The differences between wild populations and laboratory model systems have been illustrated in a study of wild and laboratory mice (*Mus musculus*), which showed that compared with laboratory counterparts, wild mice had higher antibody levels and more activated leukocytes, including T helper cells, B cells, dendritic cells and macrophages; responses were also more variable among wild mice (Abolins *et al*., 2011). Some field studies on human populations in endemic areas have been able to analyse the immunology of natural infections in resource-limited populations (reviewed in Jackson *et al*., 2009b; McSorely *et al*., 2010; Supali *et al*., 2010; Bourke *et al*., 2011), but such studies are by no means numerous.

In wild animal populations, studies of 'ecological immunity' have used a variety of global assays in order to estimate the strength of host immune responses, and shown with great effect that measures of disease resistance can be influenced by a number of factors, including maternal effects, reproductive investment, and ageing (e.g. Lozano & Lank, 2003; Palacios *et al*., 2007; Arriero *et al*., 2009). Birds are by far the most commonly studied wild populations in this respect, due to ease of capture and manipulation, and several assays for assessment of immune function are used. The most widely used is perhaps the phytohaemagglutinin (PHA) test, where PHA, a toxic protein produced by legumes, is injected into the wing web, and the thickness of the subsequent swelling measured. PHA is a mitogen to many vertebrate cell types, and provides

an indicator of the proliferative response of circulating T lymphocytes (Smits *et al*., 1999). However, it is still unclear whether the test is a useful indicator of investment in acquired immunity which is meaningfully related to host condition, or whether it is reflective of an innate response to tissue damage (Kennedy & Nager, 2006). The test has also been criticised because it is unclear how it is related to parasite-induced immune responses, which may be extremely specific in different host-parasite interactions (Owen & Clayton, 2007). These criticisms have led to the call for the use of more specific measures of immune responses, which are reflective of individual physiological state and have meaningful relationships with parasite infection intensity and/or measures of host fitness (Bradley & Jackson, 2008). It has been recommended that studies should identify the most ecologically relevant parasites, and immune responses specific to that parasite can then be assayed alongside more general measures in order to understand the host-parasite interaction, with the immunological literature as a guide (Adamo *et al*., 2004; Graham *et al*., 2011; Pedersen & Babayan, 2011).

Longitudinal studies with the potential to collect such data will be extremely important in advancing this area of research. In the first study of its kind in a natural population, Graham *et al*. (2010) assayed anti-nuclear antibody (ANA), a natural autoantibody commonly implicated in human autoimmune disease (Arbuckle *et al*., 2003; Smee *et al*., 2007), but also involved in normal immune function (Dighiero & Rose, 1999). Associations between ANA and other measure of immune function were measured in a subset of the highest ANA responders, and it was found that ANA was positively associated with immunoglobulin (Ig)G, antibodies to ribonucleoprotein, and antibodies to *T. circumcincta*, the most prevalent parasitic nematode in the population (Graham *et al*., 2010). Levels of ANA were also heritable, and were positively associated with survival of population crashes and longevity in adult females. Complex interactions with reproduction were found, indicating that ANA were negatively associated with

reproductive success in males and negatively associated with probability of giving birth in females, although in females that did give birth, lamb birth weight was positively associated with maternal ANA (Graham *et al*., 2010). These findings show that longitudinal studies of immune function can be used to reveal the importance of immunity in mediating life history trade-offs, something which has long been posited (Sheldon & Verhulst, 1996).

1.5 The Soay sheep of St Kilda

In this section, I will describe aspects of the study population and data collection relevant to the work undertaken in this thesis. More detail is provided on the relevant data collection and biology of the sheep in the introductory sections to each chapter.

1.5.1 Population history

The St Kilda archipelago of the Outer Hebrides, NW Scotland (Figure 1.1), is made up of four islands, the two largest of which are Hirta (638 ha) and Soay (99 ha). Historically, Hirta has held a human population of around a hundred from as long ago as the Bronze Age, but the population began to dwindle in the late nineteenth century due to emigration and was evacuated at the inhabitants' own request in 1930 (Campbell, 1974). Soay remained unpopulated by humans, but a population of free-living sheep (*Ovis aries*; Figure 1.2) has inhabited the island for several thousand years (Clutton-Brock & Pemberton, 2004). They resemble the earliest domesticated sheep that spread through Europe and arrived in the Scottish islands around three or four thousand years ago, and were abandoned on Soay, where, protected by steep cliffs and rough seas, they have lived freely ever since (Clutton-Brock & Pemberton, 2004). In 1932, 107 sheep

were moved from Soay to Hirta (65 ewes, 22 tups, and 20 castrates), from which the current population on Hirta has grown. The current study began in 1985, and the island population has varied between 800 and 2000 individuals during that time. Intensive study focuses on the population inhabiting an area of the island known as Village Bay, which covers approximately a third of the island's area and contains around a third of its sheep.

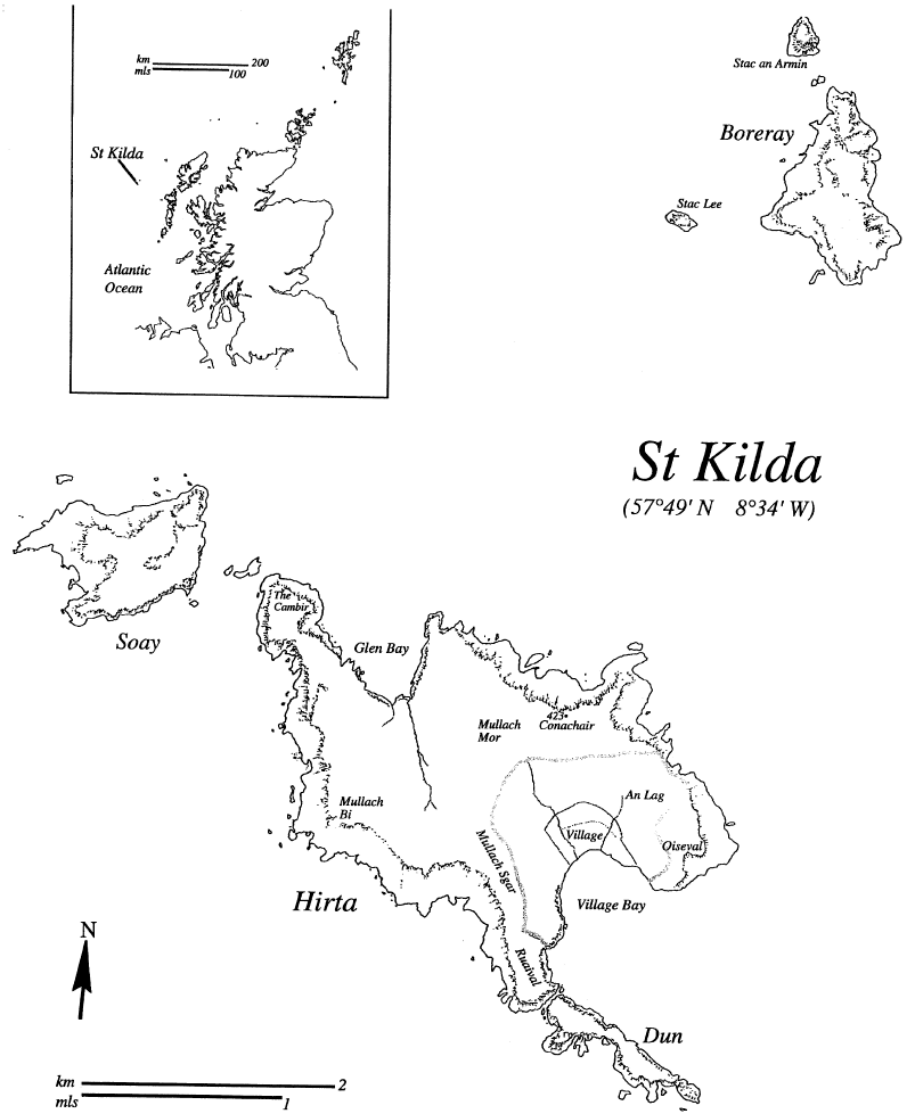

Figure 1.1: The islands of the St Kilda archipelago. The focus of the study is on individuals frequenting the Village Bay area of Hirta (outlined with grey shading). Map from Clutton-Brock & Pemberton (2004).

1.5.2 Data collection

Fieldwork takes place in three seasons (for full details, see Clutton-Brock & Pemberton, 2004). In each season, ten censuses of the Village Bay study area are conducted in order to account for the survival and location of known individuals. The first of these is from March until early May, the main purpose of which is to account for and identify sheep which have died over winter, and to catch lambs. During lambing, there is no interference with the birthing process, which is entirely natural. Every spring, around 95% of lambs born in the Village Bay population are caught, mostly within a week of birth. Each lamb is sexed, weighed, blood sampled and given an individual identification tag, and so every sheep frequenting the core study area is individually recognisable; the tag is applied by removing an ear punch which is later used for genotyping. Maternities are assigned on the basis of behavioural interactions between lambs and ewes, and paternity is assigned using a pedigree constructed using Bayesian methods in the R package MasterBayes (Hadfield *et al.*, 2006). Paternity assignment is based on a combination of microsatellite and allozyme markers; fixed effects of horn type and age are included in the model since both are associated with reproductive success; paternity is assigned to a sire if that sire is predicted in at least 80% of the samples of the posterior distribution of the pedigree (Morrissey *et al.*, submitted). The second period of data collection is in August, when around 60% of individuals in the Village Bay population are captured by driving sheep into net corrals. Morphometrics are measured in each sheep, including weight, hind and fore leg length, horn type, horn length, horn circumference, and scrotal circumference. The majority of sheep are sampled for blood and faeces, ectoparasites are counted, and females are checked for the presence of milk. In addition to this, a count of sheep across the entire island is conducted.

Finally, in November, females are in oestrus and males compete for matings, in the period known as the rut. Males are immobilized, blood sampled, and morphometric data are collected.

Figure 1.2: Soay sheep are small, relative to other wild sheep and to early domestic sheep, with females reaching up to 30kg and males up to 45kg. Top: adult female and untagged lamb, aged around three months; above: adult male, showing large, curved horns; both photographed in August 2008. Photographs courtesy of Arpat Ozgul.

1.5.3 Population dynamics and mortality

The population exhibits highly unstable population dynamics, with severe fluctuations in population size from year to year (Figure 1.3). These fluctuations are caused by annual variation in over-winter mortality (Clutton-Brock *et al*., 1991), with annual differences in weather, population density, and demography all contributing (Catchpole *et al*., 2000; Coulson *et al*., 2001; Coulson *et al*., 2008).

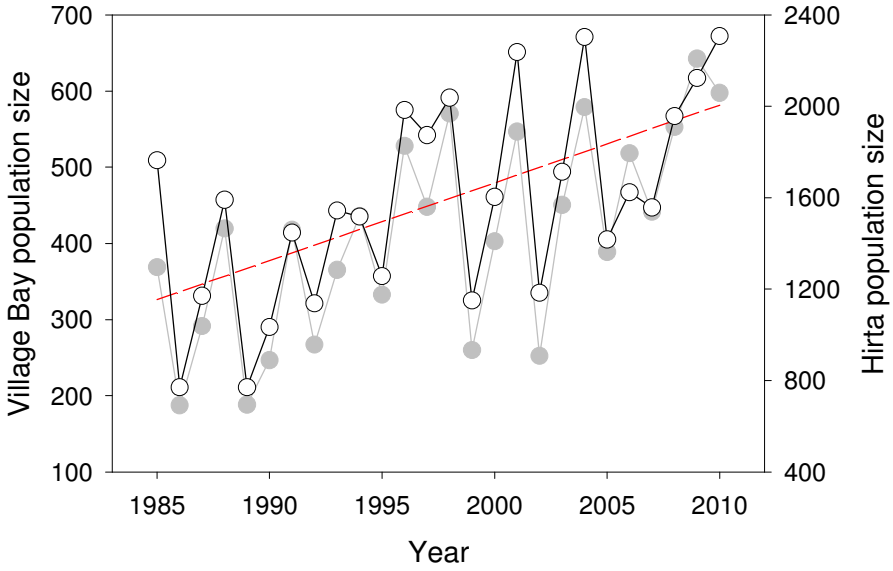

Figure 1.3: The population of the Village Bay study area (open symbols, black lines) shows marked fluctuation from year to year, although there has been an overall increase in population size since the study began (red line showing linear regression). Both of these aspects of the Village Bay population are reflected in the population size of the whole island of Hirta (filled grey symbols, grey lines).

Soay sheep are reproductively active from their first year, may reproduce in every year of their lives, and females may produce twins, and so the population increases very rapidly before exceeding carrying capacity and experiencing high mortality (Grenfell *et al*., 1992; Clutton-Brock *et al*., 1997; Clutton-Brock & Coulson, 2002). Years of particularly high mortality are

often referred to as 'crashes', and are also characterized by poor condition of survivors, who lose more body weight than in normal winters (Clutton-Brock *et al*., 1991). During crashes, the overall mortality rate can be as high as 69%, but mortality is particularly high in lambs and adult males, reaching up to 95% and 86% respectively; mortality in adult females is generally low, although can be as high as 30% in extreme years (Clutton-Brock *et al*., 1992). These winters may be considered periods of great physiological stress to individuals, with possibly far-reaching consequences for subsequent fitness.

1.5.4 Reproduction and life history

The reproductive cycle of Soay sheep begins during the rut in November. Individual females come into oestrus for between one and four days, and are regularly inspected by males until they do so (Clutton-Brock *et al*., 2004a). Males compete for dominance in head-butting contests, with heavy-bodied, long-horned males enjoying the greatest success within individual breeding seasons (Preston *et al*., 2003; Robinson *et al*., 2006). Both males and females may be sexually mature in their first year, and although rapid early development and reproduction carries potential fitness benefits, there is also a cost in terms of reduced survival if environmental conditions are unfavourable (Stevenson *et al*., 1995; Tavecchia *et al*., 2005; Robinson *et al*., 2008). The gestation period is around 21.5 weeks (Clutton-Brock, 2004), and the majority of lambs are born in April. Most are single births, but depending on the prevailing environmental conditions and female condition, 2 - 23% of litters may be twins (Clutton-Brock *et al*., 1991). Twins are born lighter and have lower subsequent fitness than singletons, but female fitness is maximized by producing twins in all but the least favourable conditions (Wilson *et al*., 2005a; 2009). Lambs suckle until early summer, when they are weaned and are essentially independent.

This period of growth is critical since survival of the first winter is highly dependent upon lamb weight entering winter (Clutton-Brock *et al*., 2004a), and maternal phenotype appears extremely important, since lamb survival is associated with maternal body weight (Clutton-Brock *et al*., 1996).

Males have shorter lifespan than females, both in terms of mean and maximum; mean longevities for adults surviving past their first winter are approximately 3.5 for males and 6.5 for females (calculated from all individuals of known birth and death date), and the oldest recorded males and females were 11 and 16 years old respectively. This difference in life span can be accounted for by the high cost of male reproductive effort, since experimentally castrated males were shown to take no part in the rut and lived for a maximum of almost seventeen years (Jewell, 1997).

1.5.5 Parasitology of Soay sheep

The sheep are infected with a range of macroparasites, with 13 species of helminth recorded in the population as well as arthropods (Table 1.1); the microparasite fauna comprises numerous species of protozoa, but bacterial and viral pathogens have yet to be characterized. The most prevalent and abundant macroparasites are the gastrointestinal strongyle nematodes *Teladorsagia circumcincta*, *Trichostrongylus axei*, and *Trichostrongylus vitrinus*. These parasites exhibit a direct life cycle which is completed in around 18 days: adult *T. circumcincta* and *T. axei* inhabit the sheep abomasum (4th stomach), while adult *T. vitrinus* inhabit the small intestine; adults shed eggs into the intestinal lumen, from where they are deposited on the pasture in sheep faeces; the eggs hatch and undergo two larval moults; the third larval stage is

ingested by grazing sheep; larvae undergo two more moults in the abomasal mucosa before developing into mature adults, mating, and producing eggs (Kassai, 1999). These species, and others, are associated with parasitic gastroenteritis (PGE), characterized by damage to the abomasal wall which detrimentally affects protein absorption; such effects have been seen in sheep dying over winter on St Kilda (Gulland, 1992).

Taxon	Species	Location
Apicomplexa	*Cryptosporidium parvum*	Small intestine
	Giardia duodenalis	Small intestine
	Eimeria spp.[a]	Small/large intestine
Arthropoda	*Melophagus ovinus*	Wool
	Damalinia ovis	Wool
Platyhelminths	*Moniezia expansa*	Small intestine
	Taenia hydatingena	Abdominal cavity
Nematoda	*Dictyocaulus filaria*	Lungs
	Muellerius capillaris	Lungs
	Teladorsagia circumcincta[b,c]	Abomasum
	Trichostrongylus axei[c]	Abomasum/small intestine
	Trichostrongylus vitrinus[c]	Abomasum/small intestine
	Capillaria longipes	Small intestine
	Strongyloides papillosus[c]	Small intestine
	Nematodirus battus	Small intestine
	Nematodirus filicollis	Small intestine
	Nematodirus helvetianus	Small intestine
	Bunostomum trigonocephalum[c]	Small intestine
	Trichuris ovis	Large intestine
	Chabertia ovina[c]	Large intestine

[a]11 species, not listed for brevity; details may be found in Craig *et al*. (2007)
[b]Previously *Osertagia*; worms previously classified as *T. trifurcata* and *T. davtiani* are recognised as *T. circumcincta* (Grillo *et al*., 2008).
[c]Strongyle nematodes, the most prevalent and abundant species; eggs from these species are counted together to provide the strongyle faecal egg count (FEC), an estimate of parasite resistance.

Table 1.1: The parasite species recorded on from St Kilda Soay sheep. Adapted from Wilson *et al*. (2004); Craig *et al*. (2007).

Although direct counting of worms in dead sheep has been undertaken on St Kilda, (e.g. Gulland, 1992; Craig *et al*., 2006), a non-invasive estimate of parasite numbers is also gained by counting parasite eggs in faecal samples using the McMaster egg counting technique (M.A.F.F., 1986). Faecal samples are collected when sheep are captured in August and throughout the year

by observing defecation of known individuals (Table 1.2). Strongyle faecal egg count (FEC hereafter) includes the three species described above as well as three others; eggs from these six species are indistinguishable by eye and so they are counted together. FEC can be tracked across individual life histories and temporal scales in order to determine the dynamics of infection within individuals and across time. It is positively associated with actual worm number in a linear fashion (Gulland, 1992; Wilson *et al*., 2004), with $r^2 = 0.425$ (Grenfell *et al*., 1995) and thus represents a good estimate of the number of worms harboured by individual sheep. FEC of individual sheep is likely to be determined in part by variation in extrinsic factors, such as spatial variation in exposure and heterogeneity in worm genetics, but these are not measurable, and are both assumed to be constant among individuals.

Despite this, several lines of evidence support the use of FEC as an individual-specific measure of host parasite resistance. The repeatability of FEC is reasonably high, estimated at 58% in females and 42% in males (Wilson *et al*., 2004), and a significant proportion of this individual variance can be attributed to host genotype, since FEC has significant additive genetic variance (Coltman *et al*., 2001a; Beraldi *et al*., 2007) and several genetic loci are associated with FEC (Paterson *et al*., 1998; Coltman *et al*., 2001b; Beraldi *et al*., 2007). Variation in FEC in the study population is associated with a number of other individual-level host factors, including sex (Wilson *et al*., 2004), age (Craig *et al*., 2008; Hayward *et al*., 2009), and behaviour (Hutchings *et al*., 2002). The high level of individual variation and covariance with phenotypic traits, coupled with the knowledge that the sheep immune response regulates both worm number and fecundity (Smith *et al*., 1985; Stear *et al*., 1995), demonstrate the value of FEC as a non-invasive estimate of parasite resistance, and a trait of individual sheep. FEC is used as an estimate of individual parasite resistance throughout this thesis, on the assumption that unmeasured environmental aspects of exposure and worm genetics are homogeneous between individuals.

	Lambs n = 778		Yearlings n = 417		Adults n = 1332	
	Prevalence (%)	Mean count (± 95% C.I.)	Prevalence (%)	Mean count (± 95% C.I.)	Prevalence (%)	Mean count (± 95% C.I.)
Strongyles	95	719 ± 50	86	407 ± 48	58	176 ± 14
Coccidia[a]	99	5788 ± 678	96	1078 ± 100	77	558 ± 44
Keds[b]	82	3.36 ± 0.24	28	0.29 ± 0.06	12	0.15 ± 0.01
Nematodirus	32	86.82 ± 11.52	0.5	0.48± 0.66	0.2	0.30 ± 0.46
Moniezia	15	NA	13	NA	6	NA
Trichuris	2	3.21 ± 1.99	0.7	0.72 ± 0.81	0.07	0.07 ± 0.15
Capillaria	0.4	0.38 ± 0.43	3	2.88 ± 1.74	0.6	0.67 ± 0.49

[a]Including all apicomplexans
[b]*Melophagus ovinus*

Table 1.2: Summary of faecal egg count (FEC) data collected during August field seasons 1993-2005, showing prevalence and mean count across age groups. From Craig *et al*. (2008).

1.6 The objectives of this thesis

The aim of this thesis is to investigate the associations between parasite infection and life-history variation in the Soay sheep of St Kilda. In chapter 2 I present the first comprehensive analysis of changes in parasite resistance across adult life, considering the effects of age and the cumulative experience of environmental conditions across the lives of individuals. This is the first longitudinal study to provide evidence of senescence in parasite resistance in a wild population, and one of the first to show that lifetime experience of adverse environmental conditions can accelerate senescence in a phenotypic trait. In chapter 3 I examine the influence of maternal effects and early life performance on parasite resistance in lambs, and show that some of these effects may persist long into adult life. In chapter 4 I test for senescence in a variety of female reproductive traits, and for effects of parasite infection on reproductive senescence. Despite finding that parasite infection has no significant effect on senescence in reproductive performance, I show that ageing patterns differ among reproductive traits, emphasising the complexity of the ageing process. In chapter 5, I perform the first analysis of natural selection on parasite resistance in the Soay sheep, complementing previous experimental work. I show that the associations between parasite resistance and host fitness change across ages and environmental conditions, identifying changes in selection across these as potential sources of the observed variation in parasite resistance typically seen in natural populations. In chapter 6, I present the first longitudinal analysis of associations between estimates of parasite infection intensity and antibody responses in a wild population. Parasite infection intensities were uncorrelated with antibody responses, but different antibody responses were correlated; there was evidence for a genetic basis to this correlation. Finally, in chapter 7, I present a general discussion of the findings.

Chapter 2

Ageing in a variable habitat: environmental stress affects senescence in parasite resistance in St Kilda Soay sheep

This chapter has been published as: Hayward, A.D., Wilson, A.J., Pilkington, J.G., Pemberton, J.M. & Kruuk, L.E.B. (2009) Ageing in a variable habitat: environmental stress affects senescence in parasite resistance in St Kilda Soay sheep. *Proceedings of the Royal Society of London B- Biological Sciences* **276**, 3477-3485.

2.1 Summary

Despite widespread empirical evidence for a general deterioration in the majority of traits with advancing age, it is unclear whether the progress of senescence is chronologically determined, or whether factors such as environmental conditions experienced over the lifespan are more important. We explored the relative importance of 'chronological' and 'environmental' measures of age to changes in parasite resistance across the lifespan of free-living Soay sheep. Our results show that individuals experience an increase in parasite burden, as indicated by gastrointestinal helminth faecal egg count (FEC) with chronological age. However, chronological age fails to fully explain changes in FEC because a measure of environmental age, Cumulative Environmental Stress (CES), predicts an additional increase in FEC once chronological age has been accounted for. Additionally, we show that in females age-specific changes are dependent upon the environmental conditions experienced across individuals' life histories: increases in FEC with age were greatest amongst individuals that had experienced the highest degree of stress. Our results illustrate that chronological age alone may not always

correspond to biological age, particularly in variable environments. In these circumstances, measures of age that capture the cumulative stresses experienced by an individual may be useful for understanding the process of senescence.

2.2 Introduction

Biological senescence is a general age-specific decline in physiological condition and fitness (Bonsall, 2006). It is manifested in a wide range of traits, from key life-history traits, such as age-specific survival and reproductive performance (Monaghan *et al*., 2008), to aspects of cellular physiology such as telomere length (Monaghan & Haussmann, 2006) and oxidative damage (Monaghan *et al*., 2009). The crux of interpreting senescence within an evolutionary framework is that the number of surviving individuals in any cohort decreases with age due to extrinsic causes of mortality, and so the strength of natural selection declines with age. Theoretically, the onset and rate of senescence can be perfectly predicted by an individual's true 'biological' age, an indicator which would predict the ageing state of an individual better than 'chronological' age (Klemera & Doubal, 2006), which equates to the time since birth in units such as days or years. However, chronological age does not take into account additional environmental factors which may influence the proximate mechanisms of ageing (Monaghan *et al*., 2008), and which may therefore contribute to an individual's biological age. It has been shown in a number of studies that conditions during early growth and development can have profound effects on fitness (e.g. Kruuk *et al*., 1999) and on the trajectory of senescence in later life (e.g. Nussey *et al*., 2007a; Reed *et al*., 2008). Environmental conditions can therefore play a large role in determining life history trajectories, particularly with reference to senescence. In this context, a metric measuring 'environmental' age, encompassing the cumulative

environmental conditions experienced by an individual across its lifespan, could aid understanding of senescence-related changes in key life-history traits, especially in free-living systems where individuals are subject to stochastic environments. 'Biomarkers of ageing', such as telomere length, hormonal changes, and a range of immunological parameters (Simm *et al*., 2008), are thought to provide alternative indicators of biological age, but studying such parameters in the wild has proven difficult. It is therefore not clear how well alternative measures of ageing describe changes in performance across an individual's lifetime.

Over the last twenty years, a growing body of work has shown that senescence is pervasive in wild populations (e.g. Jones *et al*., 2008), and occurs in a range of traits in organisms including insects (Bonduriansky & Brassil, 2005), fish (Reznick *et al*., 2004), birds (Gustafsson & Pärt, 1990; Brommer *et al*., 2007; Keller *et al*., 2008) and mammals (Beauplet *et al*., 2006; Nussey *et al*., 2006). Typically, individuals experience declines in survival probability and reproductive performance as they age. Susceptibility to infection also increases, through the process of immunosenescence, an age-specific deterioration in the efficiency of the immune system (Tarazona *et al*., 2002). This subject has received much attention in the laboratory (see Gruver *et al*., 2007), but there has been limited work in natural populations and especially in mammals (but see Festa Bianchet 1989; Festa-Bianchet 1991; Pelletier *et al*., 2005 for a notable example of a longitudinal study of helminth infection in a wild population). Previous work in wild bird populations has proven consistent with a decline in aspects of the immune system with age (Cichon *et al*., 2003; Palacios *et al*., 2007). However, such studies are, as far as we are aware, exclusively cross-sectional and as such do not account for the possibility that the observed results are due to individual differences, cohort effects, or inter-annual variation. In contrast, use of longitudinal data allows separation of within-individual change from between-individual heterogeneity (Nussey *et al*., 2008).

The Soay sheep population on St Kilda is the subject of one of the world's most intensive longitudinal studies of a free-living mammal population (Clutton-Brock & Pemberton, 2004). Data have been collected for over twenty years on population dynamics, individual life histories, parasitism, and environmental variables, and provide a unique opportunity to examine the effects of ageing on parasitism. By using individual-based longitudinal data on parasite burdens, we attempted to identify how ageing affects resistance to parasites in this population. We use three separate indicators of biological age: chronological age in years, and two alternative measures indicating an individual's cumulative experience of the environment, which we term 'environmental' age. The first of these sums the number of years of severe mortality an individual has experienced, and the second takes into account environmental conditions experienced in every year of life from birth until sampling to assess the impact of lifetime environmental experience. We also assess how the environmental conditions experienced by individuals across their life histories affect the trajectory of changes in parasitism with chronological age.

Our primary aim was to test, using longitudinal data from a free-living population, for senescence in parasite resistance and to describe age-specific changes in parasitism, with the hypothesis that individuals will experience increasing parasitism as they age. We also test for differences in age-specific parasite infection between the sexes, and predict that males will age more rapidly than females (Clutton-Brock & Isvaran, 2007). Secondly, we use chronological and environmental measures of age, and examine how they affect parasitism. Finally, we attempt to identify how age-specific changes are affected by an individual's cumulative environmental experience. We predict that individuals that have experienced poorer environmental conditions will suffer elevated parasitism compared to individuals of the same chronological age that have experienced relatively favourable conditions.

2.3 Methods

2.3.1 Study population and data collection

The feral Soay sheep population of Hirta (638ha) in the St Kilda archipelago, NW Scotland (57º49'N 08º34'W) has existed in a free-living state since 1932, when 107 sheep were moved from the neighbouring island of Soay. The current individual-based study began in 1985, since when data have been collected on a range of aspects of the population including population dynamics, individual life history and morphological traits, and parasitology, as well as a suite of environmental measures (Clutton-Brock & Pemberton, 2004). The study focuses on the Village Bay area of the island of Hirta, which contains 200-650 sheep, approximately a third of the island's population. The population exhibits unusual dynamics, with periodic over winter mortality events (population 'crashes') that result in severe reduction in population size (Figure 2.1; Clutton-Brock *et al*., 1991; Clutton-Brock *et al*., 1997). Mortality is determined by a combination of population density, demographic structure, winter weather conditions, and low food availability (Coulson *et al*., 2001; Grenfell *et al*., 1998).

The sheep are parasitized by a number of parasitic helminth species (Wilson *et al*., 2004), as well as ectoparasites and 13 species of parasitic protozoa (Craig *et al*., 2007). The most prevalent parasite species in the population are the gastrointestinal strongyle nematodes *Teladorsagia circumcincta*, *Trichostrongylus axei*, and *Trichostrongylus vitrinus*, infections of which are associated with overwinter mortality (Gulland, 1992; Craig *et al*., 2006) and loss of condition as indicated by reduced body weight (Craig *et al*., 2008). Data on infection with these and less abundant strongyle species, in the form of faecal egg counts (FECs), have been collected since

1988. The McMaster egg counting technique provides an estimate of the number of eggs per gram of faeces, and has been shown to be a good index of parasite burden in Soay sheep, both on St Kilda and elsewhere (Wilson *et al*., 2004). In our analyses, we used strongyle FEC as our response variable for estimating resistance to parasite infection.

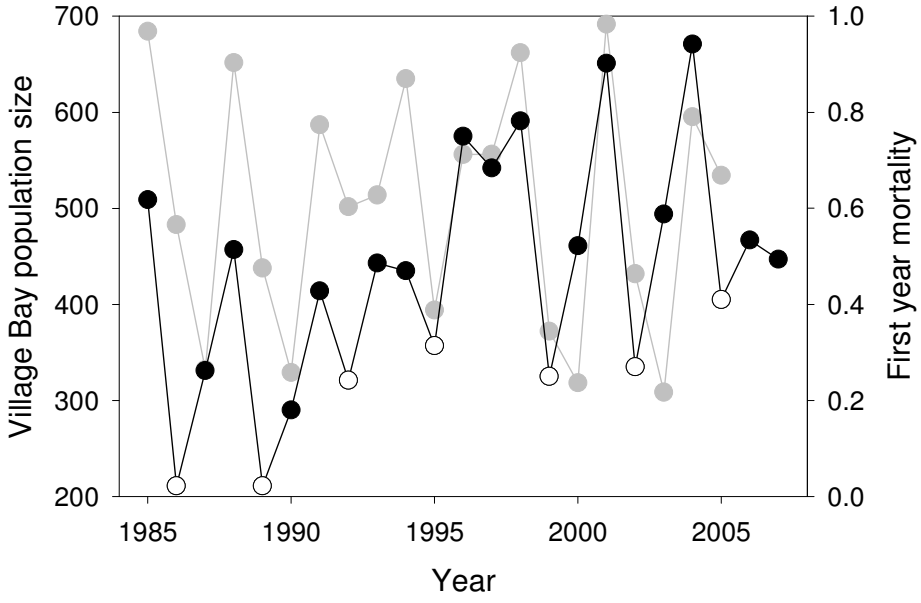

Figure 2.1: The St Kilda population inhabits a highly variable environment. The system exhibits severe inter-annual fluctuations in population density (black line), where open symbols show 'crash years', and in first year mortality (grey line), which has varied from 21% to 98% over the course of the study.

2.3.2 Data and variables

Analysis was performed on data collected between 1985 and 2006, comprising 1806 faecal samples from 227 females and 683 samples from 70 males. This does not represent the total number of FECs available, since we removed a proportion of the full dataset for three reasons.

Firstly, lambs and yearlings suffer from extremely high parasite burdens before gradually acquiring immunity (Wilson *et al*., 2004; Craig *et al*., 2006), and so we excluded all FECs collected from individuals younger than the age of two. Secondly, during the history of the project, a number of experimental administrations of anthelmintics have been made, and so any samples collected less than a year after anthelmintic treatment were excluded from our analyses. Finally, we only considered individuals which had died, and for which we had complete life-history data.

'Chronological' age in years was included in all initial models as linear, quadratic and cubic terms, in order to test for a curvilinear effect of age on FEC. We also quantified 'environmental' age, which is an individual's experience of the environment and an estimate of the amount of stress it has experienced up to the point of sampling, using two metrics. Our assumption is that conditions experienced immediately before and during a crash are more stressful than those experienced immediately following a crash, since adult female sheep show a larger reduction in body weight between autumn and March in crash years (Clutton-Brock *et al*., 1991). The first measure of environmental age was given by the number of winter population crashes an individual has survived (Figure 2.1) with more crashes equating to more stress. Secondly, we used a measure of environmental quality, E, which is the proportion of lambs of a cohort surviving for at least one year (Wilson *et al*., 2006). This value provides an indicator of environmental quality based on survival of lambs of both sexes.

Although the factors influencing lamb survival and adult survival are not identical (Coulson *et al*., 2001), factors which negatively influence lamb survival, such as density and winter weather, have similar effects on female sheep past their reproductive peak and smaller but detectable effects on prime age adult survival (King *et al*., 2006; Coulson *et al*., 2008). March rainfall is

negatively associated with survival in all age and sex classes (Catchpole *et al*. 2000). Our measure of environmental quality, E, is negatively associated with population density, winter weather variables, and March rainfall (A. Hayward, unpublished data), indicating that environmental conditions affecting adult survival and performance are similar to those affecting senescent and prime-aged female sheep. To gain a measure of environmental stress experienced by an individual, this measure was inverted to give the proportion of lambs dying within a year, and then summed from the time of an individual's birth until the time of sampling, giving a measure of environmental age, cumulative environmental stress (CES). Since this measure accumulates with, and positively covaries with, chronological age, we required an alternative measure to predict the influence of environmental conditions across the lifespan on changes in FEC. To remove the colinearity between environmental age and chronological age, we took the mean of the yearly values of inverted E, and subtracted it from each yearly value. By summing these mean-centred values from birth until the time of sampling, we obtained a measure of the quality of environment experienced over the lifespan that was age-independent, which we refer to as relative environmental stress (RES). Thus, individuals with more positive values of RES have experienced a poorer environment than individuals of the same age with more negative RES.

An individual's age at death (longevity) was included as a covariate in all of our analyses, in order to account for selective disappearance, the heterogeneity in survivorship of individuals which can produce misleading results in longitudinal analysis of age-specific traits (van de Pol & Verhulst, 2006). Intra-annual seasonal environmental conditions are likely to have significant effects on parasite infection, and so we included a number of variables to account for this possibility. Females in particular experience a peri-parturient rise (PPR), an elevation of parasite burden around the time of offspring birth (Houdijk, 2008). We considered season of sampling as

a factor with two levels: 'Lambing' (samples collected in April and May), and 'Other' (all other months, chiefly August). Population density is similarly influential, generally being positively correlated with parasitism (Morand & Poulin, 1998), and so we included Village Bay August population density (PD) and previous August population density (PPD) as continuous variables. Parasitism is likely to be influenced by climatic conditions, particularly where they have an effect on host condition and survival, as they do in this population (Milner *et al*., 1999b). The North Atlantic Oscillation (NAO) is a general measure of climatic conditions, with high values indicating warm and wet weather, and low values cool and dry weather, and is commonly used in ecological studies (Stenseth *et al*., 2003). The winter NAO is an average of the monthly NAO values for December to March (Gibraltar-Reykjavik index) and here provides a measure of the climatic conditions during the winter before sampling. Finally, to test for any temporal trend in FEC, and to account for any inter-annual variation not explained by the specific variables described above, we included year as a continuous covariate in our analyses.

2.3.3. Statistical analysis

To test for changes in FEC with indicators of age, we used generalized linear mixed-effect models (GLMMs), and all analyses were performed using the GLMM procedure in GenStat 11th edition (VSN International). We used a negative binomial error structure, in order to account for the highly overdispersed nature of parasite data, with few hosts containing the majority of parasites (Wilson and Grenfell, 1997). The negative binomial distribution is described by the mean and k, a term describing the extent of aggregation ($k = \mu^2 / \sigma^2 - \mu$), and we calculated separate values for both sexes combined ($k = 0.344$), females ($k = 0.299$) and males ($k = 0.549$), indicating that FEC is more uniformly distributed amongst males. We used a log link function,

estimated the dispersion parameter for each model, and used the conditional fitting method of Schall (1991).

In all of our analyses, we included individual identity and year of collection as random effects to account for non-independence of samples taken from the same individual or in the same year. Below, we describe a preliminary model testing for sex-specific differences in patterns of FEC with ageing, and then four subsequent models, each of which were performed on data from both female and male sheep, and which attempt to identify the effects of chronological and environmental measures of ageing.

Model 0: We pooled data for females and males, and constructed a model designed to assess the factors affecting FEC in adults in this population. We investigated sex-specific differences in variables affecting FEC by fitting sex, longevity, season, PD, PPD, NAO, year, and linear, quadratic, and cubic terms for age, as well as interactions between sex and the other variables, where parentheses indicate random effects: $FEC \sim sex + longevity + season + PD + PPD + NAO + year + age + age^2 + age^3 + sex{:}longevity + sex{:}season + sex{:}PD + sex{:}PPD + sex{:}NAO + sex{:}year + sex{:}age + sex{:}age^2 + sex{:}age^3 + (ID) + (year)$. This initial model was simplified using the method described below to a final model, which indicated that FEC followed a quadratic trajectory with age (age est. = -0.248 ± 0.069, d.f. = 1, Wald = 4.31 , p = 0.038; age² est. = 0.024 ± 0.005, d.f. = 1, Wald = 13.49, p = <0.001). There was also a significant interaction between sex and age (male est. = 0.106 ± 0.043, d.f. = 1, Wald = 6.15, p = 0.013). Inspection of the parameter estimates reveals that FEC remains effectively constant in males from ages two until four, and then increases, with the highest FEC in the oldest sheep at age 8. In females, FEC is predicted to initially decline with age, reaching a trough around the age of five or six, before subsequently increasing from the age of seven onwards. This result,

coupled with the differences between the sexes in biology, longevity, parasite aggregation and age distribution of the data, encouraged us to separate the sexes for subsequent analyses.

Model 1: Having established that age-specific changes in FEC differ between the sexes, we described the relationship between chronological age and FEC in each sex separately with a model incorporating current environmental factors and chronological age:

$$FEC \sim longevity + season + PD + PPD + NAO + year + age + age^2 + age^3 + (ID) + (year)$$

These models allowed us to fit sex-specific aggregation parameters to represent the differences in distribution of FEC between the sexes.

Model 2: Our second model assessed changes in FEC with the number of population crashes experienced by an individual, as a crude measure of environmental age, while controlling for chronological age. Note that AGE indicates the linear and quadratic chronological age terms in females, and solely the linear term in males, since these were the variables that emerged from model 1 in females and males respectively: $FEC \sim longevity + season + PD + PPD + NAO + year + AGE + crashes + (ID) + (year)$

Model 3: Thirdly, we employed our cumulative measure of environmental age, CES, as an alternative to the number of population crashes experienced, in order to further assess changes in FEC with environmental age, while controlling for chronological age: $FEC \sim longevity + season + PD + PPD + NAO + year + AGE + CES + (ID) + (year)$

Model 4: Finally, to assess the influence of environmental experience on changes in FEC with chronological age, we used a model with an interaction between RES and chronological age:

FEC ~ longevity + season + PD + PPD + NAO + year + AGE + RES + AGE:RES + (ID) + (year)

All initial models were simplified until only significant variables, or those involved in significant interactions, remained. Significance of fixed effects was assessed using Wald statistics and associated conditional p values with the appropriate degrees of freedom.

2.4 Results

2.4.1 Associations between ageing and FEC

Model 1:. In female sheep, there was a significant quadratic effect of age on FEC (est. = 0.025 ± 0.001SE, Wald = 19.55, d.f. = 1, p = <0.001; Figure 2.2), indicating a decline in FEC from the age of two until the age of five, followed by a rapid increase in later life. Male sheep, on the other hand, showed a significant linear increase in FEC from the age of two onwards (age est. = 0.127 ± 0.036, Wald = 12.54, d.f. = 1, p = <0.001; Figure 2.2). Hence, there is evidence to suggest that Soay sheep undergo a senescent decline in the ability to resist parasite infection, but that this decline contrasts between sexes, as predicted by the preliminary results from Model 0.

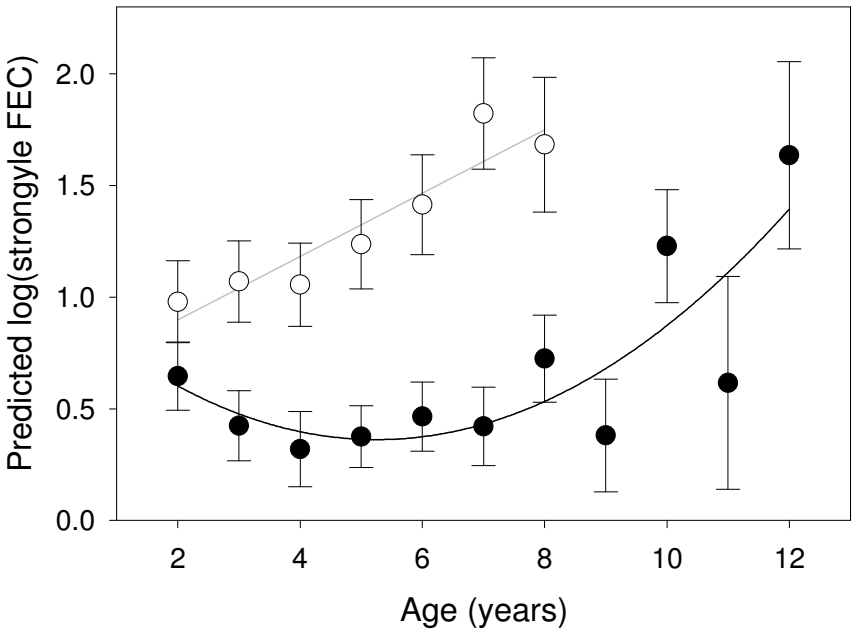

Figure 2.2: Model predictions of age-specific means and SEs of FEC. Female sheep (filled symbols, black line) experience enhanced resistance to parasitism until the age of five, and experience a subsequent decline thereafter. On the other hand, males (open symbols, grey line) show a linear increase in strongyle FEC from the age of two onwards, and maintain higher FEC than females across life.

Model 2: The final model in females incorporating number of crashes experienced showed a significant effect of number of crashes on FEC (Wald = 15.02, d.f. = 4, p = 0.005; Figure 2.3a). Thus, in a model controlling for chronological age, females experienced an increase in FEC with increasing environmental age. The effect of crashes remains highly significant if chronological age is omitted from the model (Wald = 32.17, d.f. = 4, p = <0.001). The final model for males did not show a significant increase in FEC with increasing environmental age (Wald = 4.65, d.f. = 2, p = 0.099; Figure 2.3b).

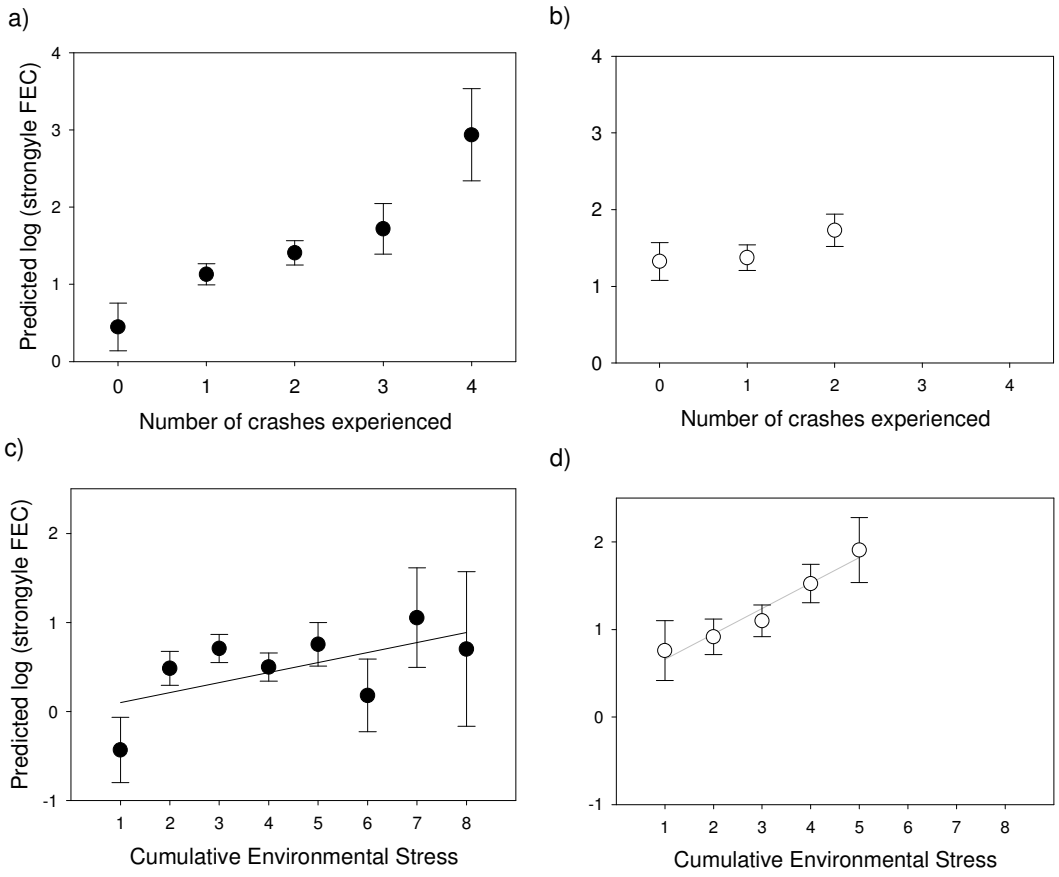

Figure 2.3: Model predicted means and SEs for age-specific changes in FEC for two measures of environmental age. a) Females show a significant increase in FEC with the number of population crashes experienced, and this effect is most marked in the oldest individuals; b) males show a non-significant increase in FEC as they experience more population crashes; c) Females show a highly significant linear increase in FEC with accumulating environmental stress; d) Males show a marginally non-significant linear increase with increasing experience of environmental stress.

Model 3: We next fitted a model with CES as a measure of environmental age, again controlling for chronological age. We found a significant positive linear relationship between CES and FEC in females, indicating that individuals that have experienced a higher degree of environmental stress suffered from increased parasitism (Table 2.1; Figure 2.3c). This effect remains if chronological age is dropped from the model (age = 1.280 ± 0.047, Wald = 7.45, d.f. = 1, p =

0.007). However, males exhibited a marginally non-significant positive effect of CES on FEC (Table 2.2; Figure 2.3d). As with the number of crashes experienced, this is suggestive of a similar process to that occurring in females, yet is not supported by statistical significance.

Variables	Estimate	S.E.	d.f.	Wald	p value
Fixed effects					
Intercept	1.193	0.128			
Longevity	-0.113	0.027	1	15.03	<0.001
Season					
Lambing	0.000	0.079	1	297.86	<0.001
Other	-1.438				
PPD	0.002	0.001	1	5.67	0.028
NAO	0.170	0.064	1	8.77	0.005
Age	-0.632	0.114	1	2.56	0.110
Age^2	0.021	0.006	1	20.87	<0.001
CES	0.723	0.173	1	17.50	<0.001
Random effects					
ID	0.500	0.079			
Year	0.136	0.068			

Table 2.1: Results of the final minimal generalized linear mixed-effects model showing the effect of environmental age on strongyle FEC in female sheep.

Variables	Estimate	S.E.	d.f.	Wald	p value
Fixed effects					
Intercept	1.513	0.195			
Longevity	-0.128	0.039	1	4.21	0.045
Season					
Lambing	0.000	0.144	1	18.50	<0.001
Other	-0.657				
NAO	0.226	0.082	1	5.54	0.026
Age	-0.056	0.105	1	12.59	<0.001
CES	0.315	0.170	1	3.43	0.065
Random effects					
ID	0.255	0.073			
Year	0.153	0.079			

Table 2.2: Results of the final minimal generalized linear mixed-effects model showing the effect of environmental age on strongyle FEC in male sheep.

A further note on these models is that parameter estimates for chronological age from models with and without CES are inconsistent across models. In female model 1, chronological age has a quadratic effect on FEC, describing a decrease and subsequent increase in FEC with age. However, in female model 3, the quadratic effect of age describes a decelerating decline with increasing chronological age (Age est. = -0.632 ± 0.114; Age² est. = 0.021 ± 0.006, Wald = 20.87, d.f. = 1, p = <0.001). While there is colinearity between these two variables, the effect of CES does not change whether or not age is accounted for, and so here is a robust indicator of changes in FEC.

Model 4: Finally, an interaction model attempting to identify an effect of environmental experience on age-specific changes in FEC yielded a significant interaction between age² and RES in females (est. = 0.052 ± 0.021, Wald = 6.36, d.f. = 1, p =0.012; Figure 2.4), indicating that the change in FEC with age changes from negative to positive with increasing stress suffered. Therefore, at low stress, FEC decreases with age, while at high stress it increases with age. The trajectory of age-specific changes in FEC with age is therefore dependent on an individual's experience of the environment over its life history. In males, the interaction between chronological age and RES was non-significant (est. = -0.125 ± 0.103, Wald = 1.49, d.f. = 1, p = 0.223), suggesting that FEC in males is independent of environmental conditions experienced over the lifespan.

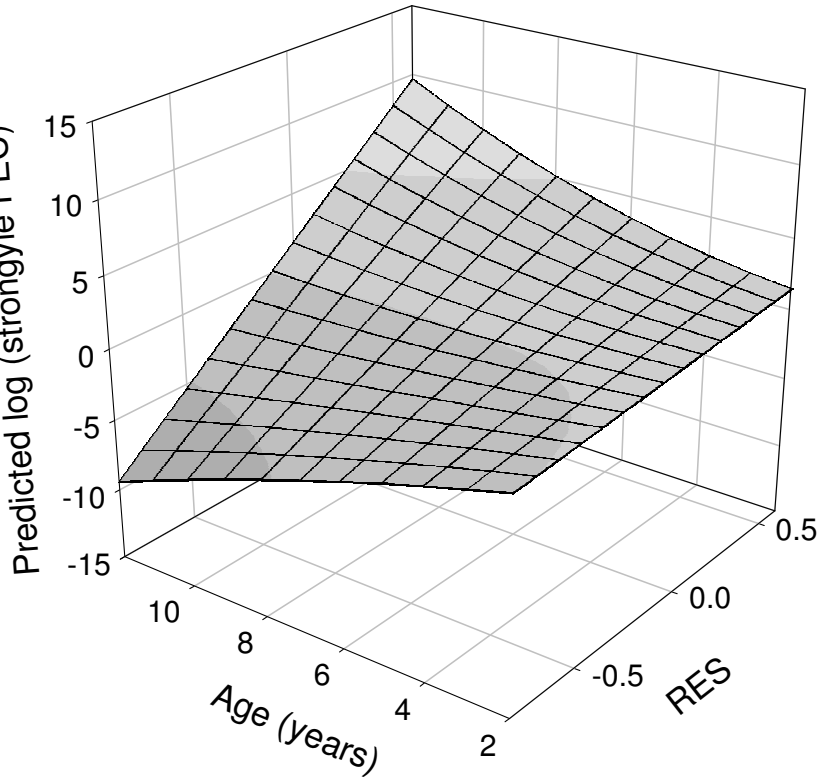

Figure 2.4: Female sheep experiencing a higher degree of environmental stress across their life spans exhibit higher FEC at a given age than female sheep experiencing lower stress. At low levels of environmental stress, FEC decreases with chronological age, but this pattern is reversed in females enduring higher levels of stress.

2.4.2 Other variables influencing FEC

Tables 1 and 2 show other variables that were found to influence FEC in all models in females and males respectively. Both sexes experienced significantly higher FEC during the lambing season and following winters with high NAO values, indicating warmer and wetter weather. Longevity was negatively associated with FEC in both females and males, indicating that longer-lived sheep generally exhibit lower FEC and justifying our attempts to control for selective disappearance. Finally, previous summer's population density was positively associated with

FEC in females but not males, indicating that transmission events occurring prior to the winter may influence worm burden during the following year. This demonstrates that specific environmental effects influence parasitism, and that simply accounting for inter-annual variation by including year as a covariate or random effect may not be sufficient in this respect.

2.5 Discussion

We have presented results showing that, as predicted by previous work on immunosenescence, feral Soay sheep experience declining ability to resist parasite infection as they age chronologically. We have also shown that an alternative measure of ageing, environmental age, also predicts an increase, and that chronological age alone is insufficient to describe senescence in this context. Finally, we have demonstrated that the nature of change in parasitism with age is highly dependent upon the environmental conditions individuals experience across their life histories.

Many life-history traits measured in wild populations can be well explained as a quadratic function of chronological age, with an improvement in the trait from early life until a peak in 'prime age', followed by a senescent decline in later life (Jones *et al*., 2008). The bulk of studies of immunosenescence either compare age classes (e.g. Saino *et al*., 2003) or show a linear decline in immunological parameters with age (e.g. Haussmann *et al*. 2005; Palacios *et al*., 2007), which therefore contrast with the quadratic form described in studies of other traits. A decrease in assayable immune parameters does not necessarily predict an increase in parasitism with senescence, since an optimal immune response is not necessarily the strongest possible (Viney *et al*., 2005), but in both female and male sheep we show that there is an increase in

parasitism with chronological age, suggesting senescence in the efficiency of the immune response to helminth infection. Moreover, it appears the age-related increase in FEC begins from age 8 in females and age 5 in males (Figure 2). This represents data on 93 different females (41% of the individual females in our dataset) and 31 different males (44% of individual males in our dataset), indicating that a substantial proportion of individuals surviving to adulthood reach an age at which they experience increasing parasite burden as they get older. This is, as far as we are aware, the first longitudinal analysis of senescence-related changes in parasite infection or resistance.

Our two indicators of environmental age, number of crashes experienced and CES, both predicted an increase in FEC, and these results also hold whether or not chronological age was included in the model. This indicates that chronological age alone does not describe senescence-related changes in FEC, and that incorporating a measure of environmental age in addition to chronological age can provide more information about the process of senescence.

By using RES as a relative measure of environmental age, we have shown that conditions experienced throughout life can have a profound impact upon age-specific changes in an important fitness trait, namely FEC, an estimate of parasite burden and resistance. Thus, when experiencing low levels of environmental stress, females showed an improved ability to resist parasites with age, as estimated by falling FEC. However, when experiencing relatively poor conditions over their lives, females showed a progressively faster increase in FEC with age. This shows that environmental conditions can have a profound impact upon rates of senescence. The results of analyses on males suggested that changes in FEC with chronological age seemed to be independent of RES. A possible explanation could be simply that we lacked the statistical power to detect any interaction, though this is unlikely given that standard errors were lower for males

than females. A second explanation is that the more rapid life history of males (Clutton-Brock *et al*., 2004a) makes them less vulnerable to the cumulative effects of environmental stress, since the majority may not live long enough to express its effects. Similarly, because of the lower life expectancy of males, they do not experience the same range of cumulative environmental stress as females and so either do not express its effects or do so only weakly.

Although we cannot identify the proximate mechanisms driving these changes, we can comment in broad terms on how cumulative exposure to environmental stress may affect parasite resistance. One possibility is that experience of adverse environmental conditions has irreparable effects on physiology that are proportional to the cumulative amount of stress suffered. For instance, limited resources in poor conditions may be shifted away from immunocompetence and into maintenance of body weight or to a developing foetus, which may explain the effects of previous population density and NAO on FEC we have shown here. Persistent experience of poor environments and parasite infection may have an adverse effect on the ability of the immune system to respond to infections in later life, as the proliferative capability of T cells becomes exhausted (Akbar *et al*., 2004, Vleck *et al*., 2007). It has also been shown that strongyle infection can cause physical damage to the sheep abomasum (Gulland, 1992), and such damage accumulated over time could have adverse effects on ability to assimilate nutrients and therefore maintain an effective immune system. A final possibility is that sheep experiencing poorer cumulative environments have faced greater exposure to parasites than other sheep of the same age, and so for instance sheep with a higher RES for a given age may simply express past exposure, rather than current state, in higher FEC. Since current state depends on past experience of environmental conditions, it would be extremely difficult to separate these effects.

This longitudinal study suggests that senescence-related changes in parasite resistance are dependent upon the environmental conditions experienced over the lifetime of an individual. We are unable here to identify the mechanistic nature of the relationship between parasitism, ageing and the immune system, and so a fruitful avenue of future research will be to characterize immunosenescence in a longitudinally monitored natural population and to relate this to actual parasite burdens. We have used a ubiquitous indicator of environmental quality, namely the proportion of first year mortality, and so identifying specific environmental variables which influence senescence-related changes provides a challenge for further research, in this and in other systems. A direct and intuitive route from the current study would be to expand the analyses herein to investigate occurrences at different stages of the life-history of individuals and their effects on ageing and parasitism. Conditions experienced during early development, either pre- or post-natally, could contribute to age-specific changes in the same way as environmental ageing. Further, parasitism during development could affect later-life changes in other life-history traits. In relatively constant environments, chronological and environmental age will be virtually equivalent, since damage or stresses caused by environmental conditions will accumulate at a constant rate. Our current results indicate that in assessing any such impacts on life histories, the environmental conditions an individual experiences across its lifespan may play a significant role in determining rates of ageing, and that these impacts will be particularly noticeable in variable environments.

Chapter 3

Maternal effects and early-life performance are associated with parasite resistance across life in free-living Soay sheep

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3.1 Summary

Maternal effects occur when the maternal phenotype influences that of the offspring in addition to the effects of maternal genes, and may have a considerable influence on offspring parasite resistance. These effects, and the effects of early levels of reproduction and parasite resistance, may persist into later life and even influence ageing rates. Here we analyse a 20-year longitudinal data set collected on a free-living population of Soay sheep, to investigate the associations between a suite of maternal phenotypic traits and early-life performance on measures of parasite resistance across life. Our results show that maternal effects are important in determining offspring parasite resistance, since lambs born as twins and those born to the youngest and oldest mothers show higher parasite burdens. We show that the association between parasite resistance and natal litter size persists into adulthood. We also show that age-specific changes in parasite resistance in males are associated with natal litter size, and that age-specific changes in females are influenced by early-life levels of reproduction and parasite infection. These results add to the growing evidence that conditions experienced by individuals

during development can have a profound influence on immediate and late-life performance and may even influence ageing.

3.2 Introduction

It is well-established that conditions experienced during pre- and post-natal development are a critical determinant of performance throughout life (Lindström, 1999). However, whilst the influence of environmental conditions and maternal effects on traits such as survival and reproductive performance are well documented (e.g. Marshall & Uller, 2007), less is known about the long-term effects of pre- and immediately post-natal conditions on immune function, particularly in natural populations. In this paper, we identify associations between maternal effects and early-life performance and parasite resistance throughout life, and investigate how these may shape individual trajectories of parasite resistance in an unmanaged population of Soay sheep (*Ovis aries*).

Maternal effects may be defined as influences of the maternal phenotype on that of her offspring, in addition to the direct effects of her genes (Rasanen & Kruuk, 2007). Maternal effects include traits expressed by the mother herself, such as weight, age, and litter or brood size, but may also include offspring traits such as birth weight. Maternal effects are a key determinant of offspring immunocompetence during early life, and transfer of maternal antibodies plays a key role in priming the neonatal immune system for infection (Carlier & Truyens, 1995), but how this relates to ecological factors is unclear (Grindstaff *et al*., 2003). Transfer of antibodies in ungulates is through colostrum during the neonate's first few suckling bouts (e.g. Pfeffer *et al*., 2005), while there is apparently no pre-natal transfer via the placenta unless the placenta is in

some way damaged (e.g. Gabriël *et al*., 2005). As well as affecting neonatal immunocompetence and fitness, maternal effects and conditions experienced during development can be associated with immunocompetence after maturity (Reid *et al*., 2006), and the influence of early-life variables on performance may even last until the terminal stages of life and affect the rate of senescence. Harsh environmental conditions during development may accelerate senescence in reproductive performance (Nussey *et al*., 2007a), as may high reproductive effort in early life (Nussey *et al*., 2006). However, whether early-life conditions affect adult immunocompetence and immunosenescence has not yet, to our knowledge, been investigated.

The free-living population of Soay sheep on the island of Hirta, St Kilda, NW Scotland, presents an opportunity to investigate associations between maternal effects and early-life performance and parasite resistance. Individuals may live to extreme old ages: up to eight or nine in males and into the early teens in females, facilitating study of life history strategy and ageing. Previous work has demonstrated the importance of maternal effects in this population. Birth weight is a major determinant of fitness, being positively associated with both neonatal survival (Clutton-Brock *et al*., 1992) and lifetime breeding success (Wilson *et al*., 2005a). Lambs born to middle-aged mothers are heavier than those born to young or old mothers (Lindström *et al*., 2002), and thus maternal age can have a profound impact upon lamb fitness, with middle-aged females raising offspring most successfully. In addition to extensive life history data, the Soay sheep project has collected data on parasite infection since 1988, with fourteen helminth parasite species recorded (Wilson *et al*., 2004). The most prevalent species are the gastrointestinal strongyle nematodes *Teladorsagia circumcincta, Trichostrongylus axei* and *Trichostrongylus vitrinus* (Craig *et al*., 2006). Strongyles may have a major impact on individual fitness in this population, since they are negatively associated with survival of lambs and yearlings (Gulland, 1992), and it has been shown that susceptibility to infection and mortality is potentially

associated with inbreeding (Coltman *et al*., 1999a). A negative association between both strongyle count and adult body weight has also been demonstrated (Craig *et al*., 2008, Coltman *et al*., 2001a), although there is no detectable association between parasite infection and adult survival (Craig *et al*., 2009). The relationship between immunological parameters and actual parasite numbers in such populations is far from clear, although there is evidence that parasite-specific IgA is negatively correlated with worm numbers or fecundity in both domestic (Henderson and Stear, 2006) and free-living (Coltman *et al*., 2001b) sheep populations, and so there is some empirical basis for the assumption that parasite load is negatively associated with immunocompetence. Quantitative genetic analyses have shown a substantial contribution of maternal genetic effects to parasite resistance in both domesticated (Stear *et al*., 2009) and unmanaged (Coltman *et al*., 2001a) populations of sheep. The observation of a decline in the magnitude of these associations with age (Bishop *et al*., 1996) is consistent with analyses of maternal effects on other traits (e.g. Wilson *et al*., 2005b).

In this study, we investigate associations between maternal effects and early-life variables on a measure of parasite resistance in juvenile and adult sheep, with two major aims. Firstly, we test for associations between maternal traits and parasite resistance in young sheep, and assess the degree to which these associations persist into later life and affect ageing. We predict that traits positively associated with juvenile performance, such as large size, good condition, small natal litter size, and prime maternal age, will be associated with higher parasite resistance in young sheep. We also predict that the strength of these associations will be lower in adults than in young sheep. Secondly, we aim to investigate the associations between early-life levels of parasite resistance and reproduction, and parasite resistance in later life. We predict that parasite resistance in early life will be positively associated with that in late life, and that high early reproductive effort will be associated with reduced parasite resistance in late life.

3.3 Methods

3.3.1 Study population

The Soay sheep population inhabiting the Village Bay area of the island of Hirta in the St Kilda archipelago, NW Scotland (57º49'N 08º34'W), has been the subject of an individual-based study since 1985, which has collected data on births, deaths, morphometrics, reproductive performance, environmental variation, and parasite infection (Clutton-Brock & Pemberton, 2004). Data on infection with gastrointestinal strongyle helminths, the group of parasites which are most significantly associated with fitness in the population, are collected in the form of faecal egg counts (FECs). In our data set, and from here onwards, 'strongyle FEC' refers to a mixed species count comprising *Teladorsagia circumcincta*, *Trichostrongylus axei*, *Trichostrongylus vitrinus*, *Chabertia ovina*, *Bunostomum trigonocephalum*, and *Strongyloides papillosus* (Wilson *et al*., 2004). Despite changes in the species composition of the strongyle FEC between seasons and with age, the first three species on the above list constitute the vast majority of adult strongyles (Craig *et al*, 2006). The McMaster egg counting technique has been shown to be a good index of actual parasite burden in Soay sheep, both on St Kilda and elsewhere (Wilson *et al*., 2004). We present here analyses of associations between maternal effects and early-life performance and strongyle FEC at different stages throughout the lifespan.

3.3.2 Data and variables

To perform our analyses effectively, we separated individuals into six different age and sex subsets. Table 3.1 defines the six subsets, indicates the structure of the data in each, and gives

the structure of initial models used to analyse each subset. Our first subset consisted of lambs, and since the amount and distribution of data for males and females are comparable, we analysed the sexes together. In subsequent analyses of older individuals, we analysed the sexes separately because of differences in the distribution of FEC between the sexes, and disparities in the data structure and the variables of interest. The splitting of our data set into subsets may result in an increase in the type II error rate (falsely accepting the null hypothesis of no association), but we feel the subdivisions enhance our ability to investigate our questions of interest. All sheep in our data set had died and so were ones for which we had complete life-history data.

Population variables

The population is characterized by fluctuating environmental conditions, which have the potential to influence individual performance and levels of parasitism (Clutton-Brock *et al*., 1996; Wilson *et al*., 2004). We included the following environmental variables in all models.

i) *Prior population density (PPD)*: The Village Bay total population size in the August of the year prior to faecal sampling was included as a covariate.

ii) *Winter North Atlantic Oscillation (NAO)*: We included the mean NAO index (Lisbon-Reykjavik) for December-March in the winter prior to sampling, providing a large-scale measure of global climatic conditions.

iii) *Temporal trends (YEAR)*: To test for any trend in FEC over the study period, year of sampling was included as a fixed-effect covariate.

iv) *Natal heft (HEFT):* The Village Bay population may be divided into three units, or hefts, which differ in survival, recruitment and dispersal rates, probably due to variation in habitat quality (Coulson *et al*., 1999). We included natal heft as a fixed factor with three levels to test for any differences in FEC between sheep born in different areas.

v) *Season*: The peri-parturient rise (PPR), an increase in parasite burden in mothers around the time of offspring birth, occurs with a peak on the day of parturition, which occurs in April and May (Wilson *et al*., 2004). Where relevant, we included season as a factor with two levels, Lambing (April and May) and Other (all other months). Note that seasonality is not relevant for lambs, since faeces only contain strongyle eggs at around 45 days at the earliest (Wilson *et al*., 2004), and so no counts from lambs are collected during April and May.

Individual variables

i) Sex: Male sheep generally experience higher parasite burdens than females in this population (Wilson *et al*., 2004) and so in lambs, where we analysed the sexes together, sex was included as a fixed factor with two levels.

ii) *Foreleg length (LEG) and condition*: We included LEG as a measure of skeletal body size. We estimated body condition as residuals of a regression of body mass on leg length, to give a measure independent of skeletal size (foreleg length and condition correlation est. = 0.000 ± 0.146). All measurements were taken in the August of the year of faecal sampling.

iii) *Age at sampling (AGE):* Adult sheep show an increase in parasite burden as they age which is exacerbated by experience of poor winter conditions (Hayward *et al*., 2009), and so where relevant we included age and its quadratic.

iv) *Longevity:* Including longevity in models tests for a positive association between lifespan and the trait of interest, and also accounts to some extent for selective disappearance of individuals of different lifespan when analysing ageing (Nussey *et al*., 2008). A significant effect of age in the presence of longevity would suggest that the association of the response variable with age is important even after accounting for the fact that individuals sampled at high ages may represent

a special subset of the population. In all models presented below, the significance of other terms in the model was not affected by removal or addition of longevity.

Maternal effects

Here we introduce maternal traits which may be associated with offspring FEC.

i) *Natal litter size (LS)*: Sheep are born in a litter of either one or two, and we included the litter size into which an individual was born as a fixed-effect factor with two levels.

ii) *Birth weight (BWT)*: We included BWT as a fixed-effect covariate. 95% of individuals in the study population are captured within a week of birth. Since lambs gain weight very quickly during these first few days, we corrected for age at capture by performing a regression of birth weight on capture age in days, and used the residuals of birth weight as our explanatory variable.

iii) *Maternal age (MA)*: Linear (MA) and quadratic (MA^2) terms were included in all models.

iv) *Maternal Faecal Egg Count (MFEC):* We included MFEC collected in the August before lamb birth to assess the correlation between FEC in mother and offspring. Including MFEC in models of adult FEC caused model instability, and so we excluded MFEC from analysis of adult sheep.

Early-life performance

As well as potential maternal effects, we considered a number of variables measured during the early part of life which could influence FEC in adults.

i) *First summer FEC (FSFEC)*: FEC has been shown to be repeatable within individuals (Wilson *et al*., 2004), and so we may predict that individuals with high FEC in early life would have high FEC later on in life. For yearlings and older classes, we tested the associations between FEC

collected during the first summer (around four months of age) as a covariate on later measures of FEC.

ii) *First year reproduction (FYREP)*: For female yearlings, we included a binary factor indicating whether or not females attempted to reproduce in their first spring: depending on environmental conditions, 20-80% of females give birth aged twelve months (Clutton-Brock *et al*., 2004b).

iii) *Cumulative reproductive success (CRS):* In analysis of adult females, we included the total number of lambs produced by a female from birth until sampling (CRS) as a covariate, in order to test for a correlation between total reproductive effort and FEC.

iv) *Early-life fecundity (ELF) and age at first reproduction (AFR)*: As discussed above, early-life reproductive performance is associated with later-life performance, and so we tested for an association with late-life FEC in senescent females by summing the number of offspring produced until the age of five (ELF). To control for the fact that this may depend upon the timing of first reproductive event, we included AFR as a fixed-effect factor, with females making their initial breeding attempt aged 1, 2, or older.

3.3.3 Statistical Analysis

To investigate the response of FEC in associations with maternal and early-life performance in sheep of different age and sex classes, we used generalized linear mixed-effects models (GLMMs). All analyses were performed in GenStat 11th Edition (VSN International). To account for the high overdispersion in our FEC data, we used penalized quasi-likelihood estimation and a negative binomial error structure. The negative binomial is characterized by the mean μ, variance σ^2, and aggregation parameter k, which was calculated separately for all subsets ($k = \mu^2$

/ $\sigma^2 - \mu$). We used a log link function, and the dispersion parameter was estimated separately for each model, and we used the conditional fitting method of Schall (1991).

In our longitudinal study, individuals may be sampled repeatedly within years, and across multiple years throughout their lives. In order to account for the non-independence of samples taken from the same individual and in the same year, we included individual identity (ID) and year of sampling (YEAR) as random factors. Similarly, individuals born to the same mother are non-independent, and so maternal identity was included as a random effect in all models. Finally, in analyses of adults we also included a random effect of birth year (BYEAR) to account to some extent for the between-cohort differences in conditions seen across years in the population. In lambs birth year and year of sampling are equal, and in yearlings they are confounded, and so we did not include BYEAR in these models.

Table 3.1 summarises the data structure of each age and sex subset, and indicates which of the above variables was included in the initial model used to analyse variables associated with strongyle FEC. As well as main effects, we tested for the impact of maternal effects and early-life performance on ageing rates by adding interactions between Age and early-life variables of interest. Initial models were simplified by progressively removing non-significant terms in the order of least significance. Significance of each term was assessed as when added last to the model, using conditional p values calculated from Wald statistics associated with the appropriate degrees of freedom. Wald tests are an approximation of the likelihood ratio test (LRT), and although Wald tests do make stronger assumptions, this may not be as much of a problem when assessing fixed effects (Bolker *et al*., 2009). The LRT is also more unreliable for small to moderate sample sizes (Pinheiro and Bates, 2000), and is not appropriate when using quasi-likelihood estimation as we do here (Bolker *et al*., 2009).

Subset	Description	N	IDs	Years	Fixed effects assessed	Random effects fitted
Lambs	Individuals yet to reach April 1st in the year after birth	1366	783	20	Longevity + Sex + LEG + Condition + HEFT + LS + PPD + NAO + YEAR + MA + MA^2 + MFEC + BWT + (Sex x LS) + (Sex x MA) + (Sex x MA^2) + (Sex x MFEC) + (Sex x BWT)	ID + YEAR + Maternal ID
Yearling males	Males yet to reach April 1st in the second year after birth	334	178	19	Longevity + Season + LEG + Condition + HEFT + LS + PPD + NAO + YEAR + MA + MA^2 + MFEC + FSFEC + BWT	ID + YEAR + Maternal ID
Yearling females	Females yet to reach April 1st in the second year after birth	648	128	19	Longevity + Season + LEG + Condition + HEFT + LS + PPD + NAO + YEAR + MA + MA^2 + MFEC + FSFEC + BWT + FYREP	ID + YEAR + Maternal ID
Adult males	Males living past April 1st in the second year after birth	654	107	19	Longevity + Season + LEG + Condition + HEFT + LS + PPD + NAO + YEAR + MA + MA^2 + MFEC + FSFEC + BWT + AGE + (AGE) x (LS + MA + MA^2 + BWT + FSFEC)	ID + YEAR + Maternal ID + BYEAR
Adult females	Females living past April 1st in the second year after birth	1889	231	21	Longevity + Season + LEG + Condition + HEFT + LS + PPD + NAO + YEAR + MA + MA^2 + MFEC + FSFEC + BWT + AFR + CRS + AGE + AGE^2 + (AGE + AGE^2) x (MA + MA^2 + FSFEC + BWT + AFR)	ID + YEAR + Maternal ID + BYEAR
Senescent females	Females living past April 1st in the fifth year after birth	567	103	16	Longevity + Season + LEG + Condition + HEFT + LS + PPD + NAO + YEAR + MA + MA^2 + MFEC + FSFEC + BWT + AFR + ELF + AGE + (AGE) x (LS + MA + MA^2 + FSFEC + BWT + AFR + ELF)	ID + YEAR + BYEAR

Table 3.1: Summary of the six age and sex subsets which we performed analyses on. In each case the response variable is strongyle FEC. The table contains a definition of the criteria for inclusion in each subset, the data structure of each subset, and the fixed and random effects included in initial GLMMs in each subset. N = number of observations; IDs = number of individuals; Years = number of years.

3.4 Results

3.4.1 Lamb faecal egg count

A number of maternal effects were associated with lamb FEC in the manner we predicted in the Introduction (Table 3.2). The strong negative association between FEC and longevity suggests that even in early life, individuals that are ultimately long-lived had significantly lower levels of parasite infection as lambs. This appears to be largely due to selective mortality of individuals experiencing higher FEC in early life, since on restricting analysis to lambs surviving into adulthood the parameter estimate becomes marginally non-significant (-0.033 ± 0.22, Wald = 3.64, d.f. = 1, p = 0.058). The marginally non-significant association with sex indicated that male lambs had a tendency to have higher FEC than female lambs (Figure 3.1a), and a positive association with PPD suggested that FEC in lambs was higher when the previous winter's population was larger. Both LEG and condition were negatively associated with FEC (Figure 3.1b), indicating that larger lambs and lambs in better condition experienced lower parasite burdens, but there was no association with BWT (0.028 ± 0.050, Wald = 0.31, d.f. = 1, p = 0.576). We detected a positive association with litter size (Figure 3.1a), indicating that twins had higher FEC in their first summer than singletons. The association between the quadratic of maternal age and FEC suggested that lambs born to middle-aged mothers had the lowest FEC, and that those born to younger and older mothers had higher FEC. The significant interaction between maternal age and sex indicated that the increase in FEC in lambs with mother's age was greater in male lambs than in females (Figure 3.2). We detected no relationship between MFEC and lamb FEC (est. = -0.000048 ± 0.000290, Wald = 0.03, d.f. = 1, p = 0.868).

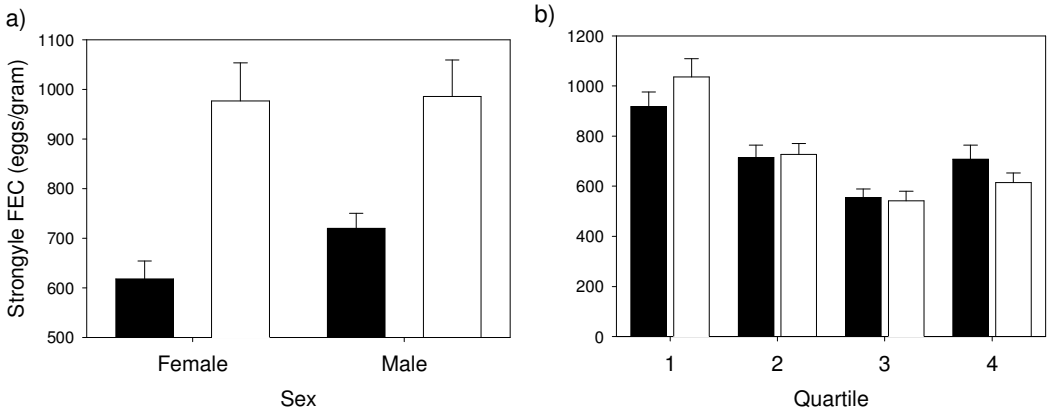

Figure 3.1: Variables significantly associated with FEC in lambs: a) singleton lambs (filled bars) have lower FEC than twins (open bars) but males and females do not differ significantly and there is no interaction; b) FEC is negatively correlated with leg length (filled bars) and body condition (open bars), divided into quartiles. Bars indicate means of raw data ± 1SE.

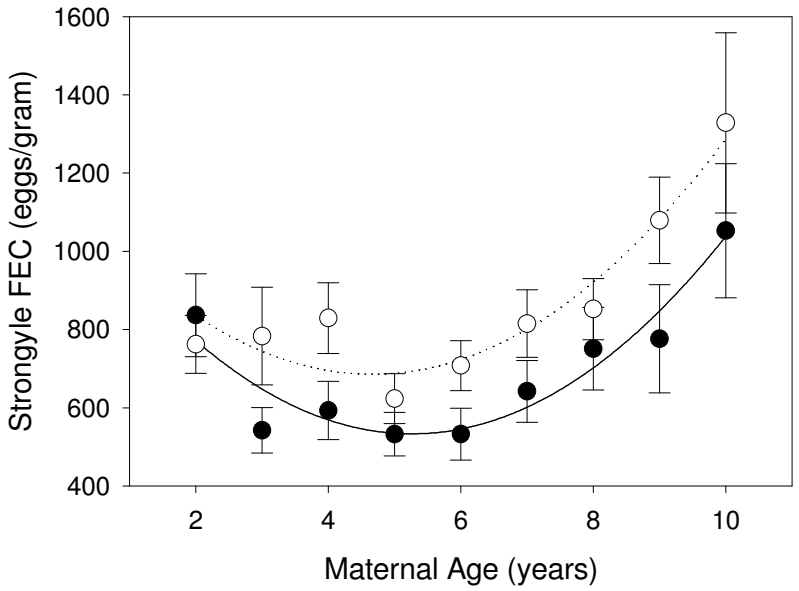

Figure 3.2: The significant main effect of maternal age on lamb FEC for each sex. The significant interaction between maternal age and sex on FEC in lambs shows that FEC in male lambs (open symbols, dotted line) increases more rapidly with maternal age than does that of females lambs (filled symbols, solid line). Points indicate means of raw data ± 1SE.

Variable	Estimate	S.E.	d.f.	Wald	p value
Fixed effects					
Intercept	1.707	0.114			
Longevity	-0.450	0.012	1	44.48	<0.001
Sex					
Female	0.000		1	2.91	0.089
Male	0.197	0.065			
PPD	0.003	0.001	1	8.20	0.009
Leg	-0.012	0.005	1	27.06	<0.001
Condition	-0.078	0.033	1	18.82	<0.001
Litter size					
1	0.000		1	5.19	0.023
2	0.194	0.085			
Maternal age	-0.173	0.054	1	0.00	0.994
Maternal age²	0.012	0.004	1	7.98	0.004
Sex x Maternal age					
Female	0.000		1	7.53	0.006
Male	0.060	0.022			
Random effects					
ID	0.237	0.034			
Year	0.187	0.073			
Maternal ID	0.009	0.020			

Table 3.2: Results from the final GLMM analyzing strongyle FEC in lambs, showing aspects of maternal phenotype and early-life performance that explain significant variation in sheep of both sexes yet to reach their first winter. See Table 1 for details of data distribution and sample sizes.

3.4.2 Yearling faecal egg counts

In male yearlings, only season was associated with FEC, with sheep suffering from higher FEC during Lambing (Other est. = -1.174 ± 0.127, Wald = 85.43, d.f. = 1, p = <0.001). We found no relationship between longevity, PPD, NAO, LEG, condition, BWT, LS, MA, MA², MFEC, or FSFEC and FEC (p = >0.100). Female yearlings also experienced higher FEC during Lambing (Other est. = -1.726 ± 0.101, Wald = 295.05, d.f. =1, p = <0.001). Both LEG (est. = -0.037 ± 0.008, Wald = 20.67, d.f. = 1, p = <0.001) and condition (est. = -0.407 ± 0.096, Wald = 17.81, d.f. = 1, p = <0.001) were negatively associated with FEC in female yearlings, but we found no associations with longevity, BWT, LS, MA, MA², MFEC, FSFEC, or FYREP (p = >0.100).

3.4.3 Adult male faecal egg counts

The results for adult male FEC suggested that natal litter size was the only maternal effect with a strong association with FEC (Table 3.3). As in lambs, longevity was negatively associated with FEC. The positive relationship between age and FEC confirms previous findings which have shown that male Soay sheep experience increasing FEC from the start of adulthood at age two (Hayward *et al*., 2009). The association with LS showed that adult males born as twins have higher FEC than those born as singletons, and there was a significant interaction between age and twin status, indicating that males born as twins showed a faster increase in FEC with age than those born as singletons (Figure 3.3). We detected no association between FEC and LEG, body condition, BWT, LS, MFEC, or FSFEC ($p > 0.200$), and a marginally non-significant association with MA^2 (est. = 0.019 ± 0.011, Wald = 0.077, d.f. = 1, $p = 0.077$).

Variable		Estimate	S.E.	d.f.	Wald	p value
Fixed effects						
Intercept		1.052	0.118			
Longevity		-0.118	0.036	1	4.32	0.039
Age		0.072	0.044	1	9.07	0.003
Litter size						
	1	0.000		1	6.10	0.016
	2	0.365	0.130			
Age x Litter size						
	1	0.000		1	6.80	0.009
	2	0.189	0.072			
Random effects						
ID		0.034	0.070			
Year		0.121	0.058			
Maternal ID		0.089	0.075			
Birth year		0.012	0.022			

Table 3.3: Results from the final GLMM analyzing strongyle FEC in adult males, showing aspects of maternal phenotype and early-life performance that explain significant variation in FEC of male sheep that have survived at least two winters. See Table 1 for details of data distribution and sample size.

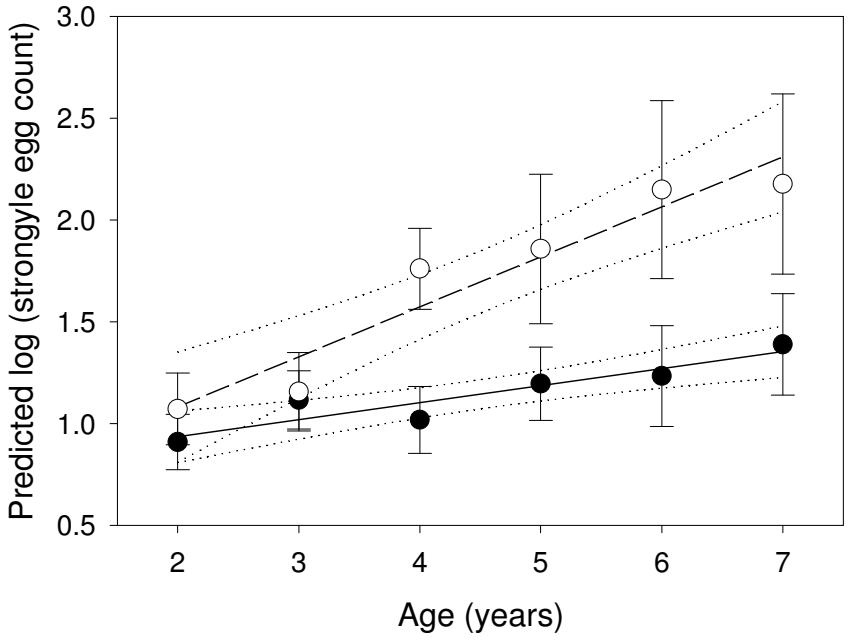

Figure 3.3: Adult males born as twins (open symbols, dashed line) show a more rapid increase in FEC with age than those born as singletons (filled symbols, solid line). Dotted lines indicate 95% confidence intervals. Plot shows predicted means ± 1S.E. from the final adult male model.

3.4.4 Adult female faecal egg counts

The final model suggested that both maternal effects and early-life performance were associated with strongyle FEC in adult females (Table 3.4). We found a negative association with longevity, and found that females experienced higher FEC during the lambing season and following years of higher population density. As seen in lambs and female yearlings, LEG and body condition were both negatively associated with FEC. As expected, FEC was a quadratic function of age, with the model predicting the lowest FEC in middle-aged females. In contrast to the findings in lambs and adult males, and having controlled for LEG and condition, adult females born as twins had significantly lower FEC than those born as singletons. Finally, the association with AFR suggested that females which began reproduction early had higher FEC than those which deferred reproduction until their second or third year.

Variable		Estimate	S.E.	d.f.	Wald	p value
Fixed effects						
Intercept		1.608	0.115			
Longevity		-0.045	0.023	1	28.28	<0.001
Season						
	Lambing	0.000		1	403.17	<0.001
	Other	-1.554	0.077			
PPD		0.003	0.001	1	12.28	0.003
Leg		-0.049	0.010	1	4.29	0.039
Condition		-0.480	0.084	1	43.17	<0.001
Age		-0.290	0.080	1	1.60	0.206
Age²		0.025	0.007	1	13.38	<0.001
Litter size						
	1	0.000		1	5.98	0.016
	2	-0.344	0.154			
AFR						
	1	0.000		2	13.94	0.001
	2	-0.166	0.124			
	3	-0.844	0.233			
Random effects						
ID		0.314	0.058			
Year		0.063	0.037			
Maternal ID		0.003	0.005			
Birth year		0.022	0.025			

Table 3.4: Results from the final GLMM analyzing strongyle FEC in adult females, showing aspects of maternal phenotype and early-life performance that explain significant variation in FEC in females that have survived at least two winters. See Table 1 for details of data distribution and sample size.

3.4.5 Senescent female faecal egg counts

Strongyle FEC in senescent females was associated with maternal effects, and results also suggested that changes in FEC with age were dependent upon early-life performance (Table 3.5). FEC in senescent females was lower in sheep that were ultimately longer-lived, was higher during the lambing season, and showed a linear increase with age. As seen in adult females, senescent females born as twins experienced lower FEC than those born as singletons. Though neither of the main effects of FSFEC or ELF were significant, both interactions with age were. The interaction between age and FSFEC suggested that older females that experienced heavier infections in early life showed a faster increase in FEC as

they aged in later life (Figure 3.4a). The interaction between age and ELF predicted that females with low reproductive performance experienced an increase in FEC with age in late life, while sheep with high performance in early life showed a decrease in FEC with age (Figure 3.4b).

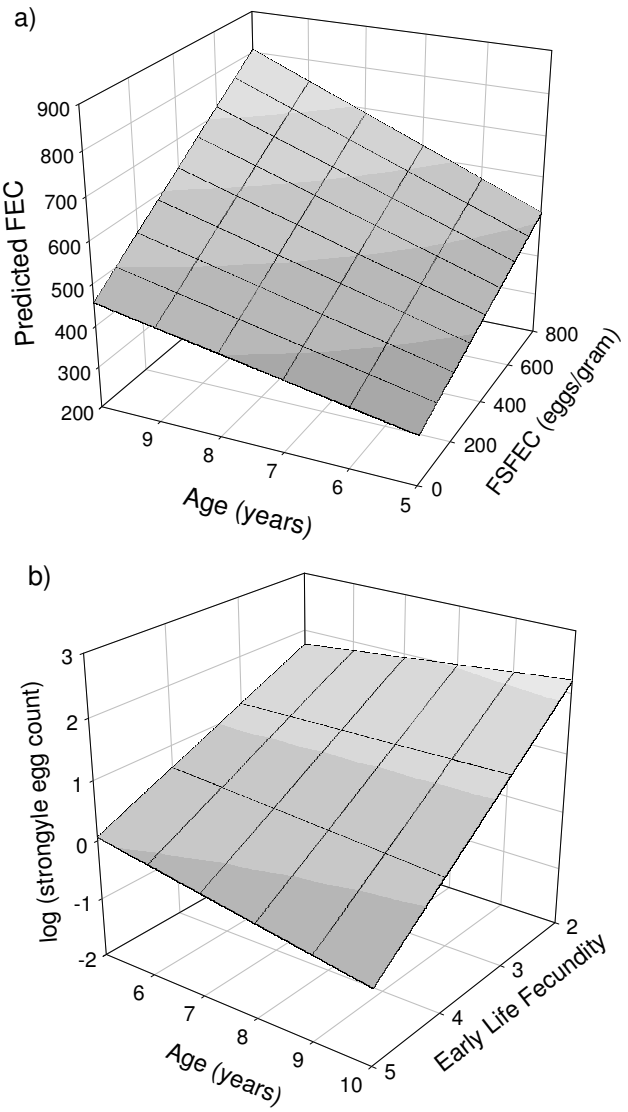

Figure 3.4: Model predictions of early-life variables associated with age-specific changes in FEC in senescent females: a) senescent females which experience high FEC in their first summer are predicted to experience a more rapid increase in FEC in late life than those experiencing low FEC in early life; b) females with high fecundity in early life are predicted to show decreasing FEC in later life, while those with low fecundity show increasing FEC. Note that axes have been pivoted to view the data most effectively, and that as a result, Age increases from right to left in a), but from left to right in b).

Variable		Estimate	S.E.	d.f.	Wald	p value
Fixed effects						
Intercept		1.568	0.142			
Longevity		-0.141	0.047	1	5.21	0.025
Season						
	Lambing	0.000		1	145.00	<0.001
	Other	-1.789	0.138			
NAO		0.303	0.072	1	10.68	0.018
Age		0.261	0.051	1	30.10	<0.001
Litter size						
	1	0.000		1	5.38	0.022
	2	-0.628	0.258			
First FEC		1.986×10^{-4}	3.171×10^{-4}	1	0.37	0.548
ELF		-0.145	0.075	1	2.68	0.107
Age x FFEC		4.695×10^{-4}	1.566×10^{-4}	1	7.24	0.007
Age x ELF		-8.342×10^{-2}	3.600×10^{-2}	1	5.37	0.022
Random effects						
ID		0.188	0.084			
Year		0.047	0.056			
Birth year		0.054	0.059			

Table 3.5: Results from the final GLMM analyzing strongyle FEC in 'senescent' females, showing aspects of maternal phenotype and early-life performance that explain significant variation in FEC in females which have survived five winters and are considered to be of 'prime age' or older. See Table 1 for details of data distribution and sample size.

3.5 Discussion

In this paper we have presented results showing significant associations between maternal effects and early-life performance and a measure of parasite infection in an unmanaged mammal population. In particular, we have shown that these associations are present not only immediately after birth and during early development, but may persist for many years into an individual's life. Below, we describe the associations found, suggest interpretations for them and highlight areas worthy of further investigation.

3.5.1 Maternal effects

Our results demonstrate that aspects of maternal phenotype are associated with FEC across the life history of individuals.

Natal litter size: Natal litter size influenced FEC in all age and sex classes with the exception of yearlings. Lambs born as twins exhibited higher FEC than those born as singletons, a result consistent with the lower lifetime fitness of twins, which is largely due to their lower birth weight (Wilson *et al*., 2005a), and increased first winter mortality (Clutton-Brock *et al*., 1992; Jones *et al*., 2005). Given the possible increase in type I error rate potentially caused by multiple testing due to splitting the data and despite a sample size of 1366, we note that caution may be attached to a p-value of 0.023. However, the relatively large parameter estimate (Table 3.2) suggests that the result is of biological significance. A potential explanation for this result is that twins receive less nourishment from their mother, since prolonged suckling provides enhanced parasite resistance in lambs, possibly due to a positive effect on weight gain (Iposu *et al*., 2008). However, by conditioning on LEG and body condition, we can be confident that the higher FEC in twins is not simply due to their being smaller or in worse condition (although this may also be relevant), but to some additional factor. Higher reproductive effort in mothers of twins may result in higher maternal susceptibility to parasites, and so lambs born as twins may therefore inhabit a more infective immediate environment. Alternatively, maternal care not related to provisioning of nutrients may be implicated, since producing twins is costly, particularly when environmental conditions are poor (Clutton-Brock *et al*., 1996). Immunosuppression during pregnancy is required to avoid the mounting of an immune response against a female's own offspring (Theodorou *et al*., 2007), and so this, coupled with the demands of raising two lambs, may mean that mothers of twins are poorer at providing protective antibodies (e.g. Pfeffer *et al*.,

2005). It has been shown in domesticated sheep that lambs of larger litters have lower serum immunoglobulin levels, and that immunoglobulin concentration is positively associated with lamb fitness (Christley *et al*., 2003). The behaviour of the lamb itself may also be implicated, since lambs of larger litters are slower to stand and suckle (Dwyer & Morgan, 2006), and so may consume less colostrum (Nowak & Poindron, 2006) which is only available for a short period. This result was replicated in adult males, in which we also found a more pronounced increase in FEC with age in males born as twins, and a less pronounced increase with age in singletons, even after correcting for LEG and condition. A p-value of 0.016 with a sample size of 654 may be a cause for caution in interpreting this, although a large parameter estimate of 0.365 and a highly significant interaction with age adds confidence to our conclusion that this association persists until long after sheep are under direct influence of their mother.

The results from adult and senescent females provide further evidence for a persistent correlation between litter size and adult FEC, but in the opposite direction to that in males and lambs, suggesting that females born as twins actually show lower FEC than those born as singletons. This is a surprising result, since individuals born into larger litters are commonly observed to be lighter at birth, which has been shown to translate into reduced lifetime fitness (e.g. Wilson *et al*., 2005a). It is possible that females born as singletons enjoy greater reproductive success, and trade this off against reduced immune investment. However, we found no association between FEC and CRS, suggesting that sustained reproductive investment over time is uncorrelated with FEC. The association also persists if longevity is removed from the model (twin est. = -0.309 ± 0.155, Wald = 4.89, d.f. = 1, p = 0.028), indicating that the correlation is not due to the selective survival of twins with high parasite resistance; the association also persists if LEG and condition are removed from the model. Female twins may be of lower fitness than singletons, and so may opt to invest in parasite resistance and survival rather than reproduction, which may explain their lower

FEC. This observation will not hold true in lambs, since there is no investment in reproduction and so this trade-off is not necessary. Male adults may be expected to invest less in maintenance than females and to invest heavily in weight gain to enhance reproductive success, potentially explaining why this may not be observed in adult males. However, investigating this possibility is beyond the scope of the current work.

Maternal age: In our analyses of lamb FEC, we showed that lambs of middle-aged females have lower FEC than lambs of younger and older mothers. If younger and older mothers are less able to provide nutrition to lambs, their lambs may begin grazing earlier than lambs of prime-aged ewes, ingest parasite larvae at an earlier age, and thus experience elevated FEC. However, since lambs may nibble grass from as early as the first week of life, it is difficult to discern when grass makes up a significant proportion of a lamb's diet. It may be that, as has been shown previously (Hayward *et al*., 2009), prime-aged females have lower FEC, and so their lambs may be raised in a less infectious immediate environment, whereas older and younger females have higher FECs and hence lambs developing in infective environments. However, splitting this relationship by sex, we found that the increase in FEC with increasing maternal age was greater in male lambs. It has been shown that rearing male lambs is more costly in terms of survival in years of high mortality (Clutton-Brock *et al*., 1996) and that reproductive costs are higher in the youngest and oldest sheep (Tavecchia *et al*., 2005). Thus, an alternative explanation is that younger and especially older females may be unable to provide as well for their demanding male lambs, and so those lambs experience higher FEC. Since LEG and condition are accounted for, this result, coupled with the associations with litter size we have found, are consistent with limitations on post-natal provisioning of antibodies leading to lower parasite resistance and higher FEC.

Birth weight: We found no association between FEC and BWT in any subset of the population. To investigate whether this was due to the association with current condition

masking an association with BWT, we repeated all analyses without LEG and condition. In yearlings and adults of both sexes, and in senescent females, BWT was still non-significant when LEG and condition were excluded from the model ($p = >0.170$ in all cases), but in lambs, there was a significant association with BWT (est. = 0.068 ±0.034, Wald = 8.76, d.f. = 1, $p = 0.003$). It therefore appears that current condition is more important than size at birth in all and sex subsets. Many previous studies have identified positive correlations between body size or condition and parasite resistance (e.g. Coltman *et al*., 2001a). The relationship between condition and parasite infection is likely to exhibit positive feedback, with poor condition increasing susceptibility to infection, which further reduces condition through reduced nutrient assimilation and voluntary reduction in food intake (Stear *et al*., 2003).

Maternal FEC: We also found no association between maternal FEC and that of their lambs. This is somewhat surprising, given that previous studies have reported a low but significant heritability for FEC in this population (Coltman *et al*., 2001a, Robinson *et al*., 2009). However, we did not have previous August FEC for even half of mothers, and so possibly lacked the power to detect any such associations. Dividing our data into subsets reduced our effective sample size further, and potentially increased the type II error rate.

3.5.2 Early-life performance

Reproduction: The negative relationship between immunity and reproductive effort is well-documented (Lochmillar & Deerenberg, 2000). However, it is also true that traits more generally associated with reproductive performance such as body size are positively associated with parasite resistance (Coltman *et al*., 2001a; Robinson *et al*., 2009). A lack of any association between FYREP and FEC in female yearlings provides no support that, in this instance, young sheep pay a cost of reproduction in terms of increased parasite load.

However, the periparturient rise associated with reproduction peaks on the day of lambing (Wilson *et al*., 2004), and by the time the majority of faecal samples are collected, in August, females have weaned their offspring (Clutton-Brock, 2004).

In adult females we found a negative association between FEC and AFR, suggesting that sheep that commence reproduction in their first year have higher FEC than those which begin in their second year, while sheep which reproduce for the first time in their third year have the lowest FEC. Life-history trade-offs between early- and late-life variables have been widely demonstrated (e.g. Nussey *et al*., 2006), and it may be that sheep which reproduce earlier trade off parasite resistance in adulthood for immediate reproductive investment. Parasite resistance may be another of the many factors which influence variation in reproductive strategies of individuals in this population (Stevenson *et al*., 2004).

In senescent females we found no correlation between ELF and FEC. However, we did find a significant interaction between age and ELF which indicated that sheep with high reproductive output during early life experienced a decrease in FEC with age, while those which reproduced less experienced an increase. This suggests a positive association between reproductive performance and parasite resistance, and is consistent with our previous findings (Hayward *et al*., 2009), since sheep which are fecund in early life are likely to be in good condition or to have experienced favourable environments, and so may age more healthily than less fecund individuals in poorer condition. The variety of associations between reproduction and FEC indicate that the relationship between reproductive investment and parasite resistance may be extremely complex.

Parasite resistance: Also somewhat surprising is the lack of any association between first summer FEC and subsequent FEC in either yearlings or adults, since the repeatability of FEC across all ages is around 0.58 in females and around 0.42 in males (Wilson *et al*., 2004).

However, it could be that much of the repeatability is due to differences in traits such as body size and condition. Although we failed to find any association between adult FEC and first summer FEC in senescent females, we found a significant interaction with age, consistent with a more rapid increase in FEC with age in individuals which experienced higher FEC in their first summer than those which experienced lower FEC. This is an interesting result in the light of previous work, which has shown that the late-life increase in FEC with age in adult females is accelerated in those females which have experienced higher environmental stress over their lives (Hayward *et al*., 2009). It is thus possible that the damaging effects of parasites experienced earlier in life, or the exhaustion of T cell repertoires due to previous infections, could potentially accelerate the ageing process (Gruver *et al*., 2007). However, the lack of any such association in adult females makes drawing firm conclusions from this impossible, especially since the comparison is being made between two measures of FEC collected five or more years apart.

3.5.3 Conclusions

In this study we have shown that aspects of maternal phenotype and early-life performance are associated with parasite infection levels in young sheep, and that these associations may persist throughout life. A criticism which may be levelled at these results is the splitting of the data into subsets, which could potentially increase the type I error rate (false positives) through multiple testing, and the type II error rate (false negatives) through reducing the amount of data analysed. We believe that analysing the data in this way has allowed us to effectively address our aims, and note that the majority of our results are highly significant and thus robust. As we predicted, traits positively associated with other aspects of lamb fitness were positively associated with lamb parasite resistance. In particular, low FEC was associated with middle-aged mothers and low natal litter size. These associations persisted

even when conditioned on body size and condition, results which are consistent with an influence of maternal provisioning not related to nutrition on offspring FEC. As predicted, the strength of these associations with maternal phenotype weakened with offspring age, and only litter size was associated with FEC in adult sheep. Again as predicted, we found evidence for a positive association between early life levels of parasite infection and adult FEC, and a negative association between early life fecundity and adult FEC in senescent females. Both of these results are consistent with a positive correlation between early life and adult fitness, but a negative association between AFR and adult FEC in adult females suggests a trade-off. These results are in line with the complex nature of the relationship between parasite resistance and other life-history traits. An interesting avenue of future research in natural systems would be to relate maternal antibody levels to those in offspring and to offspring parasite infection and survival, and to investigate associations with the ensuing offspring life-history. Bringing together immunological and ecological research in this manner would represent a crucial step in our understanding of immunosenescence and parasite ecology in naturally-regulated populations.

Chapter 4

Ageing patterns in reproductive traits of female Soay sheep: contributions of senescence, selective disappearance and terminal effects

4. 1 Summary

Many studies of reproductive senescence in natural populations consider reproductive traits in isolation and assume that the trait studied is representative of organism-wide senescence. However, this is unlikely to be true, because different aspects of reproductive performance have different physiological bases and therefore may show different patterns of age-specific variation. In order to better understand ageing in wild populations, more studies on multiple reproductive traits are needed and different causes of population-level patterns of ageing, including senescence, selective disappearance, and terminal effects need to be considered. Here, we investigate age-specific variation in female reproductive traits in a natural population of Soay sheep (*Ovis aries*), including traits related to offspring production, and traits related to maternal effects. We found significant declines in all traits in old age, except the probability of twinning. The influence of selective disappearance was analysed, and results suggested that mortality of individuals with low annual fecundity, offspring growth rate, and offspring birth weight all contribute to population-level patterns of ageing and could mask senescence if not accounted for. The influence of terminal effects varied across traits, but we found no evidence for individual differences in ageing rates, or for effects of body weight or parasite burden on senescence rates. These results illustrate that reproductive traits may show different ageing patterns that may be based on their diverse biological bases, and that only by studying a variety of traits can this complexity be better understood.

4.2 Introduction

Biological senescence may be defined as a decline in physiological function with age, and manifests itself as deterioration in condition and demographic parameters such as reproductive performance and survival probability (Monaghan *et al*., 2008). Evolutionary predictions regarding the onset and shape of senescence have largely been tested in laboratory populations of model organisms (e.g. Rose & Charlesworth, 1980; Partridge & Barton 1993; Promislow *et al*., 1996; Sgro & Partridge, 1999). It has previously been predicted that senescence should not be detectable in natural populations, due to high levels of premature mortality due to disease, predation, or starvation (e.g. Rose, 1991; Hayflick, 2000). However, recent work has provided evidence for age-specific declines in a variety of traits in such populations, including survival probability (e.g. Loison *et al*., 1999; Beauplet *et al*., 2006; Descamps *et al*., 2008), reproductive performance (e.g. McCleery *et al*., 2008; Bouwhuis *et al*., 2009; Rebke *et al*., 2010; Sharp *et al*., 2010), body weight (Berube *et al*., 1999; Proffitt *et al*., 2007), and immune function (Saino *et al*., 2003; Haussmann *et al*., 2005; Palacios *et al*., 2007). Interest has also grown in studying physiological mechanisms of ageing in wild populations, such as telomere shortening (Hall *et al*., 2004; Bize *et al*., 2009; Monaghan, 2010) and oxidative stress (Monaghan *et al*., 2009; Nussey *et al*., 2009b). In many cases, a single trait has been analysed in isolation, and taken to be representative of organism-wide senescence, but recent work has shown that not all traits show the same age-specific trajectories, and has revealed the complexity of the ageing process in natural systems (e.g. Nussey *et al*, 2009a; Lecomte *et al*. 2010). In this study, we describe ageing patterns in five female reproductive traits in a natural population of Soay sheep.

The evolutionary theory of senescence asserts that the strength of natural selection weakens with age due to declining survival probability caused by external sources of mortality such as

predation, accidents, and disease. Therefore, investment in early-life reproduction should be favoured, and late-acting deleterious mutations are under such weak selection that they persist, causing a decline in function in old age (Medawar 1952, Williams, 1957; Kirkwood, 1977). Theory predicts that the decline in reproductive performance should begin at reproductive maturity, since residual reproductive value declines from this point (Williams, 1957; Hamilton, 1966; Charlesworth, 1993). However, many studies have found evidence for an increase in traits such as brood or litter size following maturity, and a subsequent gradual decline in late life (e.g. McCleery *et al*., 2008; Rebke *et al*., 2010; Sharp *et al*., 2010). As well as senescence in offspring number, there is senescence in maternal effects, such as offspring size or survival; for instance, in blue-footed boobies (*Sula nebouxii*) chick size declines with maternal age, which is reflected in a decline in egg volume (Beamonte-Barrientos *et al*., 2010). This suggests that senescence affects not only female reproductive physiology, but also capacity to provision offspring with sufficient resources to ensure rapid growth and high survival prospects.

The initial increase in performance may be explained by increasing body size or weight, as is common in organisms with indeterminate growth (Sparkman *et al*., 2007), increased parental competence (Balbontin *et al*., 2007), or selective mortality of poor parents at an early age (Reid *et al*., 2003), and the subsequent decline is often interpreted as senescence. However, the use of longitudinal data sets has revealed the importance of selective disappearance, an association between lifespan and age-dependent traits, in studies of age-specific variation (Nussey *et al*., 2008). Selective disappearance can mask or overestimate variation in traits which is due to individual-level senescence, and the importance of accounting for it in analysis of ageing has recently been acknowledged (Cam *et al*., 2002; van de Pol & Verhulst, 2006; van de Pol & Wright, 2009; Rebke *et al*., 2010). However, far from being a statistical nuisance, selective disappearance may itself be an interesting process and

determining how it shapes ageing patterns may aid understanding of ageing in natural populations.

Other patterns which have been observed are significant increases or decreases in performance in the final year of life, which are known as 'terminal effects'. Individuals may sense their own demise, making a final 'terminal investment' in maximal reproductive effort (e.g. Bonneaud *et al*., 2004; Velando *et al*., 2006) or may show a sudden drop in performance in the last year of life, or 'terminal decline', which may be due to rapid senescence or to disease (e.g. Coulson & Fairweather, 2001; Rattiste *et al*., 2004). The majority of studies have analysed traits in isolation and often assumed that the pattern found is true of all traits, but recent work has shown that the shape of senescence varies across traits (Nussey *et al*., 2009a; Lecomte *et al*., 2010). To understand how senescence is associated with changes in reproductive performance, it is therefore essential to consider multiple reproductive traits with different physiological bases.

In this study, we analyse age-specific changes in female reproductive performance in a longitudinally-monitored population of Soay sheep (*Ovis aries*) on the islands of St Kilda, NW Scotland. Soay sheep are unusually precocious for an ungulate, and may be sexually active by the age of seven months, although females which breed so early are not highly successful in raising their lambs (Clutton-Brock *et al*., 1991). Soay females may also produce twins, which is costly but usually maximizes reproductive success, except in years where environmental conditions are very unfavourable and birth weights are very low (Wilson *et al*., 2009). Twins are born lighter and are less likely to survive the neonatal period than singletons (Jones et al., 2005), as are lambs born earlier in the season (Clutton-Brock *et al*., 1992). Lambs are weaned by the end of summer, which is extremely early in comparison to some other ungulates; for example, Canadian bighorn sheep (*O. canadensis*) and Rum red deer (*Cervus elaphus*) do not wean their offspring until early winter (Clutton-Brock *et al*.,

1983; Festa-Bianchet *et al*., 1998). Early weaning allows female Soays to potentially breed every year, whereas female red deer are still lactating during the rut following offspring birth, and so often skip years (Clutton-Brock & Coulson, 2002). Survival of lambs over the critical first winter, when up to 95% may die (Clutton-Brock *et al*., 1992), is strongly associated with birth weight and weight in their first August (Clutton-Brock *et al*., 1992; Milner *et al*., 1999b). Maternal provisioning is therefore a critical determinant of offspring survival and hence female reproductive success.

We present analysis of age-specific changes in five female reproductive traits: the probability of reproduction, the probability of twinning, offspring birth weight, offspring growth rate, and offspring first winter survival. We also test for senescence, and for effects of other traits on the rate of senescence. We test for individual differences in ageing rates, and the extent to which apparent senescence is due to individual changes or selective disappearance. We predict that all five traits will show initial improvement, related to increasing experience and body weight, from reproductive maturity to middle age, followed by a decline in old age due to senescence. We also predict that traits linked to the physiological mechanisms for producing offspring (probability of bearing offspring, probability of twinning) will show later, more rapid senescence and are more likely to show terminal declines than traits associated with maternal effects, since the former are related to oocyte number, which is fixed at birth and declines across reproductive lifespan in all mammals, but is unrelated to condition (Armstrong, 2001). Traits related to maternal effects (offspring birth weight, growth rate and survival) are more closely linked to maternal condition and resource provisioning, and so we expect to see a more gradual decline.

4.3 Methods

4.3.1 Study population

Since 1985, the Soay sheep population on the island of Hirta in the St Kilda archipelago, NW Scotland (57º49'N 08º34'W) has been the subject of an intensive individual-based study (Clutton-Brock & Pemberton, 2004). Around 60% of individuals in the Village Bay area of the island (population 200-700) are captured every August, and individuals are measured and weighed, with blood and faecal samples taken. Gastrointestinal parasite burden is estimated by counting parasite eggs shed per gram of faecal sample using the McMaster egg counting technique (M.A.F.F., 1986); we refer to this measure as faecal egg count (FEC). The reproductive cycle begins with the rut in November, when males exhibit increased aggression and compete for access to females (Preston *et al*., 2003). The majority of lambs are born in April, and birthing activity is monitored daily. Over 95% of lambs born in the study area are captured within a week of birth, blood sampled, tagged, and weighed to the nearest 0.05kg. Early-born lambs are less likely to survive the neonatal period, either due to premature birth or lack of palatable forage for mothers (Jones *et al*., 2005). The proportion of females producing twins varies from 2–23% (Clutton-Brock *et al*., 1992) and is positively associated with female weight (Clutton-Brock *et al*., 1997). The majority of neonatal mortality (defined as mortality occurring before October 1st in the first year of life; Jones *et al*., 2005) occurs in the first month of life (Overall *et al*., 2005), but from this point on, the majority of lambs survive to weaning, which occurs by mid-summer (Clutton-Brock *et al*., 2004a). Due to early lambing and weaning, females regain condition before the subsequent rut, and so can potentially reproduce every year (Clutton-Brock & Coulson, 2002). Despite study of many aspects of reproductive biology, no detailed analyses of age-specific changes in such traits have been performed. We set out to assess age-specific variation in female

reproductive traits, and to identify the importance of senescence, selective disappearance and terminal effects.

4.3.2 Data and variables

4.3.2.1 Reproductive traits

Fecundity traits: Annual fecundity (AF) was scored as 0 if a female was known to be alive in April, but was not observed to have given birth to a live lamb, and was scored as 1 if a female was observed to be engaged in caring for at least one lamb. If AF was scored as 1, twinning (TW) was scored as 0 if only one lamb was present, and as 1 if the female produced twins.

Maternal effects: The majority of lambs are captured within a week of birth, and since growth during this period is rapid, offspring birth weight (OWT) was calculated as the residuals of a regression of weight at capture on capture age. Over half of the lambs were recaptured and weighed in August, and offspring growth rate (OGR) was calculated as the difference between weight at capture in April and weight in August, divided by the number of days between the two measurements, to give a growth rate in kilograms per day. If a lamb was observed in the censuses in August or November, offspring survival (OS) was scored as 1; if a lamb was found dead or never seen in subsequent censuses, OS was scored as 0.

4.3.2.2 Analysis of age-specific changes in performance

In any analysis of age-specific traits using longitudinal data, consideration must be given as to how to separate within-individual changes from population-level changes (Nussey *et al.*, 2008). A number of methods have been developed which attempt to separate these processes,

which are based on the need to account for heterogeneity between individuals in lifespan, and the positive associations between lifespan and fitness traits. (e.g. Cam *et al*., 2002; van de Pol & Verhulst, 2006; van de Pol & Wright, 2009; Rebke *et al*., 2010). Here, we analyse ageing using models containing the following variables:

i) Age-specific changes: Female age was scored as the number of winters survived from the year of birth until the year of the reproductive event in question. We included age as a covariate with linear and quadratic terms where indicated.

ii) Selective disappearance: We included age at last reproduction (ALR) as a linear and quadratic covariate to assess the impact of selective disappearance (van de Pol & Verhulst, 2006). A significant effect of ALR suggests that changes in reproductive performance with age are due to between-individual heterogeneity in reproductive lifespan, while an effect of age having accounted for ALR predicts that a portion of the change is due to within-individual ageing. ALR was retained in all models, even if non-significant, in order to account for all of the variation in performance accounted for by reproductive lifespan.

iii) Selective appearance: We included age at first reproduction (AFR) as a linear and quadratic covariate, in order to test associations between the timing of onset of breeding and annual reproductive performance.

iv) Terminal effects: To investigate the possibility of sudden changes in reproductive performance towards the end of life, we included a factor for whether it was a female's last year of life (where analysing annual fecundity AF) or whether it was a female's final reproductive attempt (when the response variable was twinning, offspring birth weight, offspring growth rate or offspring survival). The observation of such an effect would suggest that changes in the terminal stages of life were due to one final rapid loss of function (decreased trait value) or one final effort (increased trait value).

4.3.3 Statistical analysis

We analysed age-specific changes in reproductive performance using Generalized Linear Mixed-effects Models (GLMMs). All analyses were performed in R version 2.10.0 (R Development Core Team, 2010), and GLMMs were implemented using the package lme4 (Bates & Maechler, 2009). Annual fecundity, twinning, and offspring survival were scored as binary response variables, and so we used a binomial error distribution and a logit link function. The likelihood was estimated using Gauss-Hermite quadrature (GHQ), a more accurate method for binomial traits than quasi-likelihood methods or the Laplace approximation (Bolker *et al*., 2009). Offspring birth weight and growth rate followed a Gaussian distribution, so we used a log link function. In all models, we included female's identity to account for individual differences in reproductive success, and year of sampling to account for variance in reproductive success between years.

4.3.3.1 Age-specific changes in reproductive performance

Firstly, we identified the age-specific trajectory of each reproductive trait in turn. In all models, we included fixed effects of female weight and strongyle FEC in the August prior to reproduction, since heavier females have higher reproductive success in a given year (Clutton-Brock *et al*., 1996), and parasite infection may have negative effects on reproductive performance (e.g. Newey & Thirgood, 2004; Hughes *et al*., 2009; but see Schwanz, 2009). Variation in reproductive performance in this population is associated with both density (Clutton-Brock *et al*., 1996) and climatic conditions (Forchhammer *et al*., 2001), and so we included population density in the August prior to reproduction (PPD), and North Atlantic Oscillation (NAO) for the winter prior to reproduction as a measure of large-scale climatic conditions (Stenseth *et al*., 2003). To assess the factors contributing to age-

specific variation, we included linear and quadratic terms for AFR, ALR and age. In analysis of annual fecundity, we also included whether it was a female's last year of life, and in analysis of twinning and offspring birth weight, growth rate, and survival, we included a factor for whether it was a females last reproductive attempt. In analysis of offspring birth weight, we also included natal litter size, lamb sex, and lamb birth date (all of which are associated with birth weight or survival of lambs; Clutton-Brock *et al*., 1992); the model for offspring growth rate was the same as that for birth weight, plus offspring birth weight as a fixed effect, and the fixed effects for offspring survival were the same as those for offspring growth rate. Sample sizes for each response variable are given in Tables 4.1 – 4.5.

4.3.3.2 Late-life senescence

The relationship between age and all five reproductive traits was described by a convex curve (see Results), with an initial improvement followed by a peak in middle age and a subsequent decline. To determine whether this late-life decline was significant, we restricted analysis to data from individuals of the age of peak performance and older for each trait, taking this value from parameter estimates of the models described above. We then repeated the above analysis, with age as a linear covariate only in order to test whether the decline with age was significant, and whether any other traits affected the rate of decline. For the response variables of annual fecundity, twinning, and offspring birth weight, we included interactions between age and weight, FEC, AFR, and last year or reproductive attempt; where offspring growth rate and survival were response variables we included these interactions and the interaction between age and offspring birth weight. We tested for individual variation in ageing rates by using random regression models which fitted an individual random effect of an interaction between individual and age (I x Age), testing for variation in individuals' slopes; this is equivalent to allowing the individual variance to vary

across ages. We compared the fit of this model with models with random effects of ID + year, ID alone, and year alone, and selected the best using a likelihood-ratio test (see below). We then tested the contribution of selective disappearance to apparent effects of age by comparing the significance and magnitude of the parameter estimate for age in models with and without ALR. Sample sizes for these models are shown in Table 4.7.

Full models were simplified by sequentially removing non-significant fixed effects in the order of least significance, as assessed by Wald z statistics in the case of binomial traits and χ^2 statistics in the case of Gaussian traits, tested against the appropriate degrees of freedom. Once a final model had been reached, significance of random effects was tested using a likelihood ratio test (LRT), where the test statistic was calculated as $-2*(LogL_2 - LogL_1)$, where $LogL_1$ and $LogL_2$ are the log-likelihoods of the full model and reduced models respectively, and with the p-value calculated from a χ^2 distribution.

4.4 Results

We found evidence for significant age-specific variation in all five aspects of reproductive performance. There were differences in the magnitude and shape of the predicted associations with age, and a number of other environmental and individual variables were associated with the different reproductive traits. Selective disappearance also exerted a strong effect over age-specific changes in some traits, but was not associated with others.

4.4.1 Age-specific changes in reproductive traits

Annual fecundity (AF): The variables significantly associated with annual fecundity are detailed in Table 4.1. Females were more successful in years following winters of low

population density, an earlier onset of reproduction was significantly associated with greater success in subsequent years, and sheep of the longest reproductive lifespan were predicted to be the most successful in a given year. There was also a terminal effect, with sheep significantly less likely to bear offspring in their final year of life. The significant association with age (Figure 4.1a) suggested an increase in AF at early ages, with a peak at 6 and a subsequent decline. There was significant individual variation ($\chi^2 = 61.37$, d.f. = 1, p = <0.001) and even more annual variation ($\chi^2 = 93.95$, d.f. = 1, p = <0.001), suggesting an additional environmental component beyond that accounted for by the fixed effects.

Variable	Estimate	S.E.	d.f.	z	p
Fixed effects					
Intercept	3.8442	0.6738	1	5.706	<0.001
PPD	-0.0035	0.0013	1	-2.754	0.006
AFR	-1.6502	0.3310	1	-4.986	<0.001
AFR²	0.2320	0.0753	1	3.080	0.002
ALR	-0.1936	0.0936	1	-2.068	0.039
ALR²	0.0209	0.0069	1	3.011	0.003
Last Year	-1.1876	0.1642	1	-7.232	<0.001
Age	0.7298	0.0727	1	10.044	<0.001
Age²	-0.0623	0.0062	1	-9.986	<0.001
Variance components					
ID	0.1860	0.0086			
Year	0.4578	0.0135			

Table 4.1: The final model describing variables significantly associated with annual fecundity AF in females of all ages. Results from GLMMs with binomial errors, with data of 3666 female life-years representing the 942 females across 24 years.

Twinning (TW): As expected, the probability of twinning was positively and significantly associated with female body weight (Table 4.2). We also found that the probability of twinning was significantly higher following years with low population density. Twinning was not significantly associated with AFR (-0.3033 ± 0.1663, z = -1.824, p = 0.068) or with terminal effects (last reproductive attempt = 0.2399 ± 0.3410, z = 0.704, p = 0.482), and the association with ALR was marginally non-significant. The association with age (Figure 4.1b) suggested a peak in probability of twinning around the age of 8. There was evidence for

significant individual variation (χ^2 = 29.17, d.f. = 1, p = <0.001), but no evidence for significant annual variation (estimate = 0.1461 ± 0.1100 S.E., χ^2 = 2.22, d.f. = 1, p = 0.137).

Variables	Estimate	S.E.	d.f.	z	p
Fixed effects					
Intercept	-6.0380	0.9780		-6.174	<0.001
Weight	0.1557	0.0386	1	4.033	<0.001
PPD	-0.0041	0.0009	1	-4.560	<0.001
ALR	-0.1150	0.0623	1	-1.844	0.065
Age	0.8595	0.2084	1	4.124	<0.001
Age²	-0.0496	0.0155	1	-3.210	0.001
Variance components					
ID	1.5292	0.0350			

Table 4.2: The final model showing variables significantly associated with probability of twinning TW in all female. Results from a GLMM with binomial errors on 2310 reproductive attempts by 569 females.

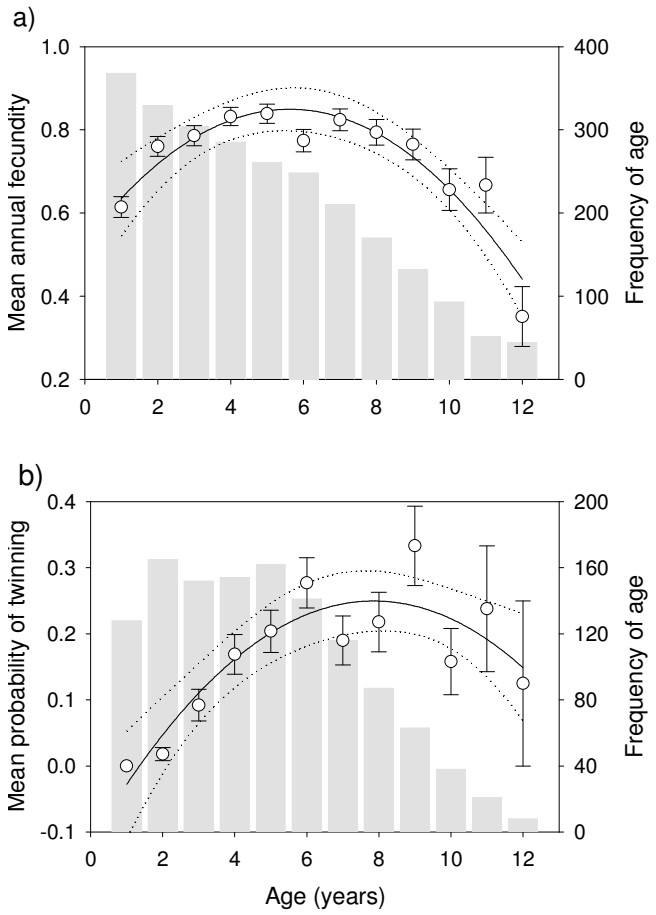

Figure 4.1: Age-specific changes in a) annual fecundity AF and b) probability of twinning TW followed a quadratic trajectory, with an improvement at early ages, a peak in middle age, and then a decline in later life. Points show mean trait values at each age ± 1 S.E.; solid black lines show quadratic curve fit to the data; dotted lines show 95% C.I.; grey bars show the number of individuals analysed at each age.

Offspring birth weight (OWT): The results for offspring birth weight suggested that male lambs were born significantly heavier than females, and that heavier females produced significantly heavier lambs (Table 4.3). We also found a significant positive association with birth date and a significant negative association with density. There was no significant association with AFR (-0.0008 ± 0.0507, $\chi^2 = 0.00$, d.f. = 1, p = 0.987) or ALR, although there was a terminal effect, with lambs born on a female's last reproductive attempt being significantly lighter than those born at any other time. The association with age (Figure 4.2a) suggested an increase in offspring birth weight as females aged towards a peak at 7, followed by a subsequent decline. There was significant variation associated with both the individual ($\chi^2 = 89.79$, d.f. = 1, p = <0.001) and annual ($\chi^2 = 22.38$, d.f. = 1, p = <0.001) variance components.

Variables		Estimate	S.E.	d.f.	χ^2	p
Fixed effects						
Intercept		-3.4837	0.4396	1		
Lamb Sex						
	Female	0.0000		1	16.06	<0.001
	Male	0.1892	0.0470			
Birth Date		0.0251	0.0033	1	57.49	<0.001
Weight		0.0354	0.0107	1	10.88	<0.001
PPD		-0.0025	0.0004	1	26.02	<0.001
NAO		-0.0879	0.0383	1	4.71	0.030
ALR		-0.0122	0.0159	1	0.59	0.443
Last Reproduction						
	0	0.0000		1	7.24	0.007
	1	-0.2230	0.0826			
Age		0.4326	0.0524	1	64.71	<0.001
Age²		-0.0316	0.0042	1	53.84	<0.001
Variance components						
ID		0.1763	0.0119			
YEAR		0.0278	0.0047			
RESIDUAL		0.5824	0.0216			

Table 4.3: Final model showing variables significantly associated with offspring birth weight OWT in females of all ages. Results are from a GLMM with normal errors performed on data from 2917 lambs born to 517 individual females across 24 years.

Offspring growth rate (OGR): Results of analysis of offspring growth rate (Table 4.4) suggested that male lambs grew significantly faster than females, and that growth rate was significantly positively associated with both birth weight and birth date. There was a significant positive association with NAO which suggested that lambs grew faster following warmer, wetter winters. There was also a significant positive association with ALR, and a terminal effect, suggesting that lambs produced on a female's final reproductive attempt grew significantly slower. The association with age (Figure 4.2b) suggested a peak around the age of 5, before a decline at older ages. The variance components associated with individual heterogeneity ($\chi^2 = 43.35$, d.f. = 1, p = <0.001) and annual variation ($\chi^2 = 67.19$, d.f. = 1, p = <0.001) were both highly significant.

Variables		Estimate	S.E.	d.f.	χ^2	p
Fixed effects						
Intercept		0.0582	0.0098			
Lamb BWT		0.0108	0.0005	1	329.10	<0.001
Lamb Sex						
	Female	0.0000		1	124.53	<0.001
	Male	0.0103	0.0009			
Birth Date		0.0002	0.0001	1	5.58	0.018
NAO		0.0032	0.0009	1	10.10	0.001
ALR		0.0007	0.0003	1	5.04	0.025
Last Reproduction						
	0	0.0000		1	16.63	<0.001
	1	-0.0063	0.0015			
Age		0.0031	0.0008	1	14.08	<0.001
Age²		-0.0003	0.0001	1	20.44	<0.001
Variance components						
ID		0.0001	0.0002			
YEAR		0.0000	0.0001			
RESIDUAL		0.0002	0.0004			

Table 4.4: Results from the final model showing variables significantly associated with offspring growth rate OGR in all females. Results are from a GLMM with normal errors, performed on data from 1105 lambs weighed at birth and in August and born to 378 females.

Offspring survival (OS): Offspring survival was significantly positively associated with birth weight and birth date (Table 4.5). There was also a significant negative association with FEC, suggesting that mothers with high FEC were less likely to wean offspring. There was a significant negative association with AFR, and a marginally non-significant tendency for higher success in sheep breeding to older ages. The terminal effect was marginally non-significant (last reproductive attempt = -0.6063 ± 0.3590, z = -1.689, p = 0.091), and the association with age (Figure 4.2c) suggested that OS increased from early ages to a peak at age 6, before a decline at older ages. Finally, variance components analysis suggested that there was no evidence for individual variation (0.0347 ± 0.1862, χ^2 = 0.02, d.f. = 1, p = 0.879), but that there was significant annual variation (χ^2 = 8.78, d.f. = 1, p = 0.003).

Variables	Estimate	SE	d.f.	z	p
Fixed effects					
Intercept	-6.7937	1.9569		-3.47	0.001
Lamb BWT	1.2223	0.1541	1	7.93	<0.001
Birth Date	0.0559	0.0166	1	3.37	0.001
FEC	-0.0012	0.0005	1	-2.14	0.032
AFR	1.6357	0.6748	1	2.42	0.015
AFR²	-0.4465	0.1611	1	-2.77	0.006
ALR	0.0859	0.0444	1	1.94	0.053
Age	0.5323	0.1715	1	3.11	0.002
Age²	-0.0473	0.0147	1	-3.22	0.001
Variance components					
YEAR	0.2781	0.0176			

Table 4.5: Final model showing variables significantly associated with offspring survival to weaning OS in females of all ages. Results are from a GLMM with analysis performed on survival data from 2736 lambs born to 578 mothers.

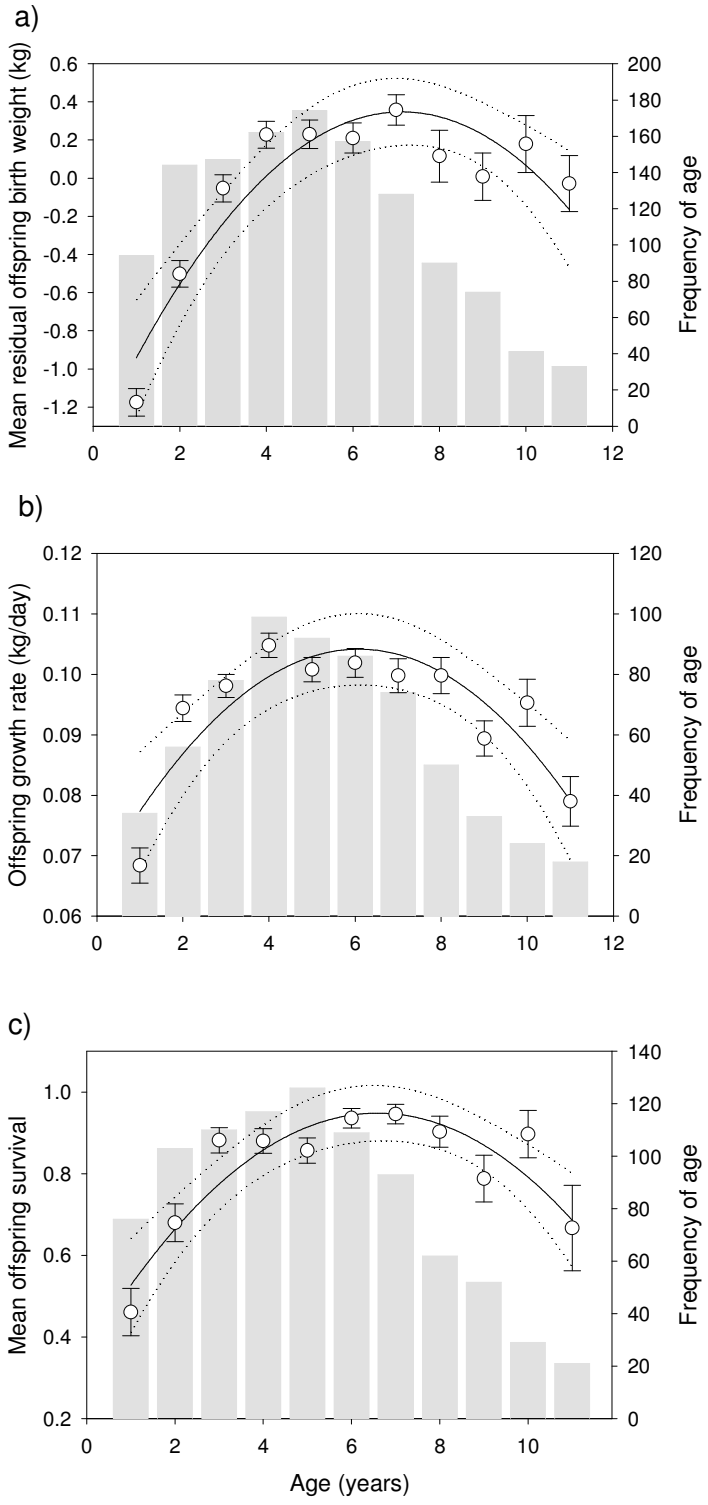

Figure 4.2: Age-specific changes in a) offspring birth weight OWT, b) offspring growth rate OGR, and c) offspring survival to weaning OS follow a quadratic trajectory with age, with an improvement in all three traits in early life, followed by a peak in middle age and subsequent significant decline at older ages. Points show mean trait values at each age ± 1 S.E.; solid lines show quadratic curves fit to the data; bars show the number of individuals analysed at each age.

4.4.2 Senescence in reproductive performance

We found evidence for significant declines after the age of peak performance in four of the five traits we analysed; only the probability of twinning did not decline significantly at older ages. The other four traits showed significant declines at late ages, but we found no statistical support for significant individual variation in the rate of change with age, since random regression models provided no better fit than models where individual variance remained constant across ages (Table 4.6). None of the interaction terms were significant, providing no statistical support for dependence of ageing trajectories upon parasite burden, weight, or AFR, and no support for changes in terminal effects across ages.

The final models for each reproductive trait are shown in Appendix A; for brevity, we comment on the effects of age-related variables only. The final model analysing annual fecundity, in sheep aged 6 and over suggested that annual fecundity was significantly higher in sheep which survived and reproduced until older ages (ALR = 2.3090 ± 0.3472, z = 6.650, p = <0.001; ALR² = -0.0895 ± 0.0197, z = -4.539, p = <0.001). There was also a significant negative association with age, indicative of senescence from the age of 6 onwards (age = -0.7443 ± 0.0620, z = -12.005, p = <0.001). In sheep aged 8 and over (the age of peak twinning rate), none of the explanatory variables were significantly associated with twinning, with no evidence of a senescent decline in individuals living to older ages (age = -0.0266 ± 0.4590, z = -0.058, p = 0.954).

The final model analysing offspring birth weight, in sheep aged 7 and over showed that there was a marginally non-significant positive association with ALR (0.0761 ± 0.0406, $\chi^2 = 3.48$, d.f. = 1, p = 0.062), and there was evidence that offspring birth weight declined significantly in the oldest females (age = -0.1399 ± 0.0293, $\chi^2 = 22.29$, d.f. = 1, p = <0.001). Analysis of

offspring growth rate in females aged 5 and above indicated that lambs grew significantly faster when born to females with longer reproductive lifespan (ALR = 0.0020 ± 0.0006, χ^2 = 12.47, d.f. = 1, p = <0.001), and there was a significant decline in lamb growth rate as females aged (age = -0.0033 ± 0.0005, χ^2 = 48.27, d.f. = 1, p = <0.001). Finally, offspring survival was not significantly associated with ALR (0.0239 ± 0.1183, z = 0.20, p = 0.840), but there was evidence for a significant decline in offspring survival with increasing age from 6 onwards (age = -0.3590 ± 0.1018, χ^2 = -3.53, d.f. = 1, p = <0.001).

Variable	Model	Random effects	LogLik	C/w	χ^2	d.f.	p
Annual Fecundity	1	(ID x AGE) + YEAR	-557.56				
	2	ID + YEAR	-560.11	1	5.10	2	0.078
	3	ID	-586.37	2	53.37	1	<0.001
	4	YEAR	-560.11	2	0.00	1	0.999
Twinning	1	(ID x AGE) + YEAR	-140.86				
	2	ID + YEAR	-140.67	1	0.38	2	0.538
	3	ID	-141.65	2	1.97	1	0.160
	4	YEAR	-144.21	2	7.09	1	0.008
Offspring Birth Weight	1	(ID x AGE) + YEAR	NA				
	2	ID + YEAR	-843.42				
	3	ID	-847.7	2	8.56	1	0.003
	4	YEAR	-888.72	2	90.60	1	<0.001
Offspring Growth Rate	1	(ID x AGE) + YEAR	1557.10				
	2	ID + YEAR	1556.90	1	0.53	2	0.765
	3	ID	1548.00	2	17.75	1	<0.001
	4	YEAR	1485.00	2	45.66	1	<0.001
Offspring Survival	1	(ID x AGE) + YEAR	-264.01				
	2	ID + YEAR	-265.32	1	2.61	2	0.271
	3	ID	-273.12	2	15.60	1	<0.001
	4	YEAR	-269.29	2	7.94	1	0.005

Table 4.6: Senescence in reproductive traits: results from variance components analysis in females aged over the peak age of each trait. Fixed effects are listed in the description of results of models for old females. χ^2 statistics indicate results of comparison of the models indicated by LRT on the appropriate degrees of freedom. Note that we were unable to test the effect of the random regression (i.e. adding ID x Age) for offspring birth weight since the model was unstable. Shaded model indicates best model selected using LRT; C/w describes the model which each model was tested against.

4.4.3 Selective disappearance and ageing

We assessed the importance of the effect of ALR on age-specific estimates by comparing the parameter estimates for age when ALR is included in or excluded from the model. The effect of age with ALR in the model describes the effect of age once the effects of selective disappearance have been accounted for, and is the estimate of individual change due to senescence; the effect of age without ALR is the population-wide pattern without differences in reproductive lifespan accounted for. Focusing on the changes occurring in sheep of post-peak age, there was considerable variation in the influence of ALR on estimates of ageing in the five reproductive traits (Table 4.7). Annual fecundity (AF) was significantly positively associated with ALR, and removal of ALR from the model resulted in a reduction in the magnitude of the estimate for age of over 60%, suggesting that selective disappearance of individuals with lower fecundity and shorter lifespan may mask the individual-level decline with age if not accounted for. ALR was also positively associated with offspring growth rate (OGR), and dropping ALR from the final model resulted in a reduction in the parameter estimate for age of 18%, suggesting that selective disappearance played a part in age-specific changes in OGR, although individual-level ageing was more important. ALR had a marginally non-significant association with offspring birth weight (OWT), and removing it from the model resulted in a 15% drop in the age estimate, indicating that individual ageing explained most of the expected change in OWT with age at the population level. ALR was not significantly associated with offspring survival (OS), and removal of it from the final model resulted in a change of only 3% in the parameter estimate for age, suggesting that most of the age-specific change in this trait was due to individual ageing.

Trait	N (IDs)	ALR estimate (± S.E.)	Age estimate with ALR (± S.E.)	Age estimate without ALR (± S.E.)	% change in age estimate
Annual Fecundity	1330 (372)	2.30 ± 0.3472***	-0.7443 ± 0.0620***	-0.2790 ± 0.0373***	-62.52%
Twinning	459 (204)	NA	NA	NA	NA
Offspring Birth Weight	670 (239)	0.0761 ± 0.0406.	-0.1399 ± 0.0293***	-0.1184 ± 0.0271***	-15.37%
Offspring Growth Rate	644 (259)	0.0020 ± 0.0006***	-0.0033 ± 0.0005***	-0.0027 ± 0.0004***	-18.18%
Offspring Survival	1174 (317)	0.0239 ± 0.1183	-0.3590 ± 0.1018***	-0.3477 ± 0.0853***	-3.15%

Table 4.7: The influence of selective disappearance on each of the five reproductive traits analysed in females older than the peak age of each trait, as determined by the parameter estimates from analysis of age-specific variation in each trait. The third and fourth columns show parameter estimates from the model described in the text; the fourth column shows parameter estimates from the same model with ALR omitted. Significance of each parameter estimates are denoted thus: *** $p < 0.001$; ** $0.001 \leq p < 0.01$; * $0.01 \leq p \leq 0.05$; . $0.05 < p < 0.1$. N (IDs) shows the number of samples for each reproductive trait.

4.5 Discussion

In this study, we investigated age-specific changes in five female reproductive traits in a natural population of Soay sheep. Our results show that there is significant age-specific variation in all five traits, but that the five traits are not described by the same ageing trajectory. Four of the five traits showed significant declines at older ages, consistent with senescence in reproductive performance, but we surprisingly found no evidence for a significant decline in twinning probability with age. The importance of selective disappearance and individual ageing also varied amongst the five traits.

4.5.1 Age-specific changes in reproductive performance

All five traits showed significant age-specific variation, and in accordance with many previous studies, all five showed improvement from the first reproductive attempt to middle age, followed by a decline during old age (e.g. Berube *et al*., 1999; Reid *et al*., 2003; Nussey *et al*., 2006; 2009a). The pattern of an initial improvement in reproductive success during the first few reproductive attempts is commonly observed in relatively long-lived animals such as ungulates (e.g. Berube *et al*., 1999; Ericsson *et al*., 2001; Nussey *et al*., 2006; 2009a), and may be attributed to female growth, increased body mass, or to experience. Soay sheep do not reach full skeletal size until the age of 2 or 3 (Figure 4.3a), and may increase in body mass until the age of 5 (Clutton-Brock *et al*., 2004; Figure 4.3b). This may explain why the youngest females showed the poorest performance in all traits apart from AF – this trait merely requires that the biological development for reproduction has occurred and is the least energetically demanding, while the other four traits require females to provide resources and care to offspring. Older females are

also more experienced and more likely to have successfully weaned a lamb previously, and it has been shown that females which have raised a lamb in one year are more likely to successfully raise a lamb in the subsequent year (Clutton-Brock & Coulson, 2002). A study of grey seals showed that second- and third- parity females of the same age as primiparous females gave birth to heavier pups (Bowen *et al*., 2006); however, number of parities was no longer significant when mass was accounted for, underlining the importance of body weight in reproductive success. In the present analysis, we have shown effects of age and body weight independently.

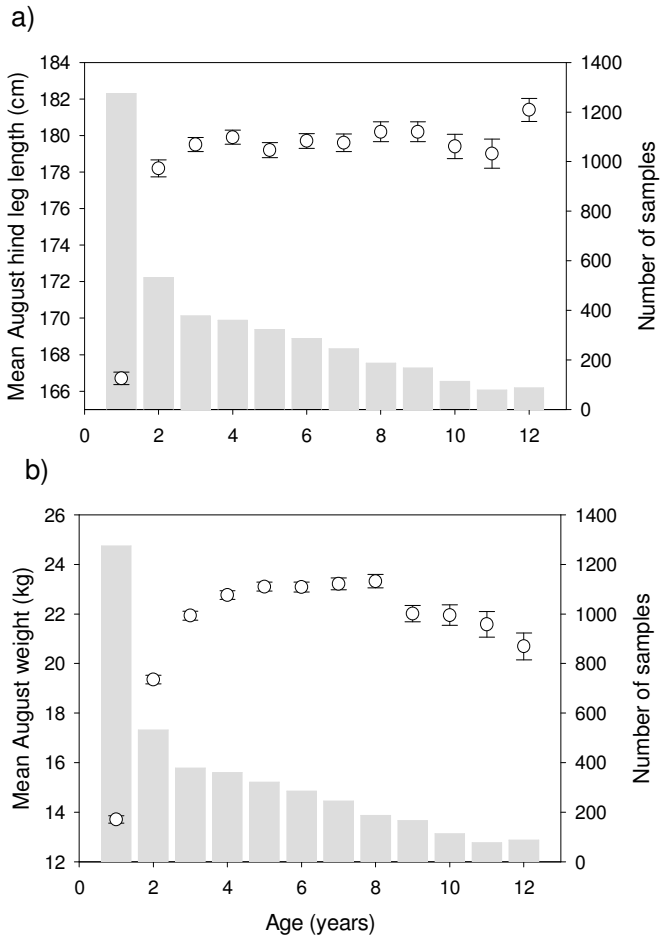

Figure 4.3: Female Soay sheep are often not fully grown by their first reproductive attempt; a) maximum skeletal size is not reached until the age of 2, and growth may continue until the age of 3; b) many females do not reach their peak body mass until the age of 4 or 5. Note that in both cases, there is likely to be some selective mortality of the smallest individuals. Points show age-specific means ± 1 S.E.; filled bars show the number of samples in each age class.

4.5.2 Early breeding and terminal effects

Annual fecundity, offspring growth rate, and offspring survival were all significantly and negatively associated with AFR, suggesting that the most successful females commenced breeding earlier in their lives. This is consistent with two non-mutually exclusive explanations. Firstly, sheep which commence reproduction at an earlier age may gain experience and so be more successful in subsequent years, and secondly, early commencement of reproduction may be associated with higher body weight or condition, which are themselves positively associated with reproductive success (Clutton-Brock *et al*., 1996; Vanpe *et al*., 2009). There was also evidence for terminal declines in annual fecundity, offspring weight, and offspring growth rate. The terminal effect on annual fecundity is consistent with females always producing a lamb if reproductive physiology is still functioning: in the last year of life, there could be a final loss of physiological function culminating in failure to reproduce and subsequent death. The terminal decline in offspring birth weight and offspring growth rate suggests that females are unable to provide offspring with resources during both the pre- and post-natal periods as effectively as they can earlier in their lives, and may be indicative of a final deterioration in performance preceding the cessation of reproductive function. These interpretations are supported by recent work which has shown that senescence in body weight in Soay sheep is very rapid, and only occurs in the final year of life (Nussey *et al*., submitted): this may be evidence of a loss of organism-wide physiological function and condition which could be predictive of imminent death.

4.5.3 Late-life declines in reproductive performance

We tested for late-life declines in performance after the predicted peak in each of the five traits. There was a significant decline in four of the traits past prime age, but we did not see this pattern in the probability of twinning. There was also no evidence for interactions between age and weight, FEC, AFR or terminal effects, indicating that these traits do not significantly influence senescence in reproductive performance. A gradual decline in traits related to maternal effects could be indicative of more gradual deterioration in condition among sheep that do reproduce at older ages. The complexity of maternal effects on offspring fitness means that age-related declines in various aspects of female physiology, occurring at different rates, could contribute to the gradual decline in offspring fitness with maternal age. Older sheep are likely to command fewer resources, for instance because of reduced feeding efficiency due to tooth wear (Gaillard *et al*., 1993; Kojola *et al*., 1998). With fewer resources, females may face a trade-off between their own condition and reproductive investment in the final years of life, and may maintain their own condition and ability to bear offspring until their very last year while having less to invest in provisioning offspring. However, there is as yet no evidence that tooth wear plays an important part in senescence in grazing ungulates (Nussey *et al*., 2007b), though one study of free-living lemurs has shown some contribution of tooth wear to reproductive senescence (King *et al*., 2005). A future analysis on tooth wear in Soay sheep could be informative in this regard.

Why was there no evidence of a decline in twinning? It has been noted before that sheep in this population aged over 6 are more likely to produce twins than are younger adults (Clutton-Brock *et al*., 1996), although this previous study split age groups into young and old adults, and so did not explore variation in old age. Even so, it was surprising that twinning did not show some

decline in the oldest females. Producing twins is associated with greater costs than producing singletons (Tavecchia *et al*., 2005), but despite lower survival of twins, there is positive selection for litter size, apart from in years of very adverse environmental conditions (Wilson *et al*., 2009). A possible explanation for the apparent lack of senescence in twinning could be that despite bearing larger litters, old females may produce lambs of lower condition than expected.

To test this hypothesis, we included interactions between age and litter size in models of offspring survival and offspring birth weight in all females and in the restricted data set of older females. A significant interaction with a negative parameter estimate would reveal that twin survival declined more rapidly with age than did singleton survival. However, when analysing offspring survival there was no significant interaction in either all females (age x litter size = 0.1871 ± 0.1023, z = 1.82, p = 0.068), or in old females (age x litter size = -0.1130 ± 0.3873, z = -0.29, p = 0.770). The same was true when offspring birth weight was used as the response variable, suggesting that the trajectory of senescence in birth weight of twins was the same as that of singletons in all females (age x litter size = 0.0304 ± 0.0294, χ^2 = 1.0625, d.f. = 1, p = 0.302) and in older females (age x litter size = -0.0160 ± 0.1007, χ^2 = 0.02, d.f. = 1, p = 0.875). Hence, there is no evidence for a trade-off between offspring number and condition in older females. Previous work in this population has shown that additive genetic variance accounts for around 11% of the total phenotypic variance in litter size, and that individual-level variation (the combined effects of additive genetic variance and the permanent environment effect) accounts for around 25% of phenotypic variance (Wilson *et al*., 2005c). This suggests that genetic differences in reproductive physiology could contribute to the lack of an age effect; for instance, if females consistently shed two ova when they come into oestrus.

4.5.4 Age at last reproduction and selective disappearance

There is substantial evidence for covariance between individual lifespan or age at last reproduction and measures of annual fitness in natural populations (e.g. Berube *et al*., 1999; Reid *et al*., 2003; Reid *et al*., 2010). In studies of ageing, failing to account for this can lead to incorrect estimates of the changes in traits occurring due to ageing. By including ALR in analysis of reproductive traits, we tested for associations between reproductive lifespan and age-specific performance, and in analyses of older females we tested the extent to which selective disappearance of individuals contributes to apparent ageing-related declines. In common with previous studies (e.g. Berube *et al*., 1999; Weladji *et al*., 2006), we found a positive covariance between ALR and annual fitness, suggesting that selective disappearance of females with low survival and low fecundity could be a large contributing factor to the observed age-specific changes. In older females, ALR was significantly associated with annual fecundity and offspring growth rate, and marginally non-significantly associated with offspring birth weight (Table 4.7), and induced 63%, 18%, and 15% changes in the parameter estimates for age respectively. This suggests that selective disappearance may mask ageing-related declines in these three traits, since individuals that survive to breed at the oldest ages are those that have the highest reproductive performance at any age.

Selective disappearance has been shown to have a large effect on clutch size in birds, with individuals laying smaller clutches dying at earlier ages (McCleery *et al*., 2008; Bouwhuis *et al*., 2009), and so it may be that some physiologically weak individuals, or individuals that cannot gain access to sufficient resources, fail to breed and die early. However, there was apparently only a negligible effect of selective disappearance on offspring survival, with the majority of the

change apparently due to individual ageing. A recent study has shown that senescence in fledgling number in common terns (*Sterna hirundo*) is largely due to within-individual ageing (Rebke *et al*., 2010); however, another study of choughs (*Pyrrhocorax pyrrhocorax*) found that age-related change in this trait was largely due to selective disappearance (Reid *et al*., 2010). This illustrates the complexity of ageing in natural populations and that patterns are likely to differ between species, even when similar traits are considered, due to variation in breeding behaviour, environment, and evolutionary history.

4.5.5 Conclusions

We have shown that age-specific trajectories and the influence of selective mortality on ageing patterns vary among reproductive traits in female Soay sheep. There was no evidence of a significant decline in the probability of twinning with age, and we were unable to satisfactorily explain why this was so using further analysis – it may be that some females exhibit a tendency to produce twins throughout life, due to genetic differences in reproductive physiology. The large effect of selective disappearance on annual fecundity suggests that some individuals in this population could be in poor physiological state from an early age, and have low reproductive success and reduced survival. There was also some evidence for effects of selective disappearance on offspring weight and growth rate, emphasizing the importance of condition in providing for offspring; individuals with sufficient resources to raise heavy lambs are those with sufficient resources to prolong their reproductive lifespan. The terminal decline in annual fecundity is consistent with the terminal decline in body weight (Nussey *et al*., submitted), with females rapidly losing body weight and the ability to either conceive or complete gestation; terminal declines in offspring weight and survival could also be linked to this. Finally, age-

specific changes in offspring survival were largely due to within-individual effects, and there were also significant ageing-related declines in annual fecundity, and offspring birth weight and growth rate in late life. These results show the complexity of ageing patterns across traits, and that there is unlikely to be a single reliable indicator trait which marks the onset of organism-wide senescence. A major limiting factor in the study of ageing in natural populations is the quality, depth, and detail of data; studies with detailed longitudinal data on multiple traits are rare, and are extremely valuable in identifying variation in ageing rates (e.g. Nussey *et al*., 2009a; Lecomte *et al*., 2010). We have also shown that the importance of selective disappearance may differ across traits, and that this can be used to make testable hypotheses about the basis of ageing in reproductive traits. Incorporating estimates of the importance of ageing, selective mortality, and terminal effects on multiple traits will aid understanding of ageing in natural populations and help identify general patterns and population-specific processes.

Chapter 5

Natural selection on a measure of parasite resistance varies across ages and environmental conditions in a wild mammal

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5.1 Summary

Parasites detrimentally affect host fitness, leading to expectations of positive selection on host parasite resistance. However, since immunity is costly, host fitness may be maximized at low, but non-zero, parasite infection intensities. These hypotheses are rarely tested on natural variation in free-living populations. We investigated selection on a measure of host parasite resistance in a naturally-regulated Soay sheep population using a longitudinal data set, and found negative correlations between parasite infection intensity and annual fitness in lambs, male yearlings, and adult females. However, having accounted for confounding effects of body weight, the effect was only significant in lambs. Associations between fitness and parasite resistance were environment-dependent, being strong during low-mortality winters, but negligible during harsher high-mortality winters. There was no evidence for stabilizing selection. Our findings reveal processes that may shape variation in parasite resistance in natural populations, and illustrate the importance of accounting for correlated traits in selection analysis.

5.2 Introduction

Individuals in natural populations are under constant threat of infection from parasitic organisms that have detrimental effects on host condition and fitness (Poulin, 2007). Infection and damage by parasites may be resisted by mounting a protective immune response, which may be condition-dependent and so should be under positive selection (Rowe & Houle, 1996; Raberg & Stjernman, 2003). These assumptions lead to the expectation of positive directional selection for parasite resistance; however, since immunity is costly (Colditz, 2008), an intermediate level of resistance which manages but does not eliminate parasites may be optimal (Viney *et al*., 2005). Individuals mounting extremely strong immune responses- and hence eliminating parasites- may actually have lower fitness because of the associated high energetic cost, while those with very low immune responses may have lower fitness because of the damage caused by a high parasite burden, ultimately generating non-linear stabilizing selection on parasite resistance and a non-linear association between host fitness and parasite infection intensity. However, estimates of the shape and strength of the association between host parasite resistance and fitness in natural populations are rare, with the majority of studies taking an experimental approach. In Kingsolver *et al*.'s (2001) review of over 2,500 estimates of the strength of selection in natural populations, not one considered selection on a measure of parasite resistance, immune phenotype, or parasite burden. In this study, we analyse the selection pressures acting on a measure of parasite resistance in a free-living population of Soay sheep (*Ovis aries*).

Experimental work in natural populations, where individuals are treated to remove parasites and compared with untreated individuals, has typically shown that parasite-free individuals show higher survival or reproductive success (e.g. Devevey & Christe, 2009; Knowles *et al*., 2010).

These studies reveal a causal link between parasites and host fitness, but because they typically compare treated versus untreated individuals, they may only reveal the costs of parasite infection, but not the associated costs of parasite resistance which would be indicated by non-linear associations between host fitness and parasite resistance. These associations are only detectable when more than two treatment levels are compared, or when the full range of natural variation in infection intensity is explored (e.g. Stjernman *et al*., 2008). Recent studies have also highlighted the importance of environmental heterogeneity and age structure in determining patterns of selection in natural populations (Rausher, 1992; Robinson *et al*., 2008; Wilson *et al*., 2009). However, non-experimental studies of associations between parasites and host fitness typically involve sampling or culling over a single selection event (e.g. van Oosterhout *et al.,* 2007; Hughes *et al.,* 2009), and it is difficult to infer from such studies whether associations between fitness and phenotypic traits change with environmental conditions or age. Longitudinal studies of selection on parasite resistance in wild populations are rare, but are necessary in order to gain a true picture of natural selection.

In this study, we estimated selection on a measure of parasite resistance in a free-living population of Soay sheep on the island of Hirta, St Kilda, Scotland. The population lacks predators or interspecific competitors, yet experiences fluctuations in population size of up to 60%, due to variation in winter mortality dependent on previous population size, weather, and food availability (Coulson *et al*., 2001). Body weight is a strong predictor of survival, and in years of very high mortality there is strong selection on body weight, with only the heaviest individuals surviving (Milner *et al*., 1999a). The sheep are infected with a variety of parasite species, the most prevalent of which are the gastrointestinal strongyle nematodes, largely made up of the species *Teladorsagia circumcincta*, *Trichostrongylus axei* and *Trichostrongylus vitrinus* (Wilson *et al*., 2004; Craig *et al*., 2006). An estimate of individual parasite resistance is

gained from counting strongyle eggs shed in sheep faeces; eggs from these and three other strongyle species are indistinguishable by eye, and so are counted together as the strongyle faecal egg count (FEC hereafter). FEC is strongly correlated with actual worm burden in Soay sheep (Gulland, 1992; Grenfell *et al*., 1995; Wilson *et al*., 2004), and although it may be influenced by unmeasured variation in exposure and worm genetics, which are assumed to be homogeneous among individuals, it is repeatable across years, with sheep identity accounting for 58% and 42% of variation in individual FEC in females and males respectively (Wilson *et al*., 2004). A significant proportion of the among-sheep variance in FEC is due to host additive genetic variance (Coltman *et al*., 2001a; Beraldi *et al*., 2007), and several loci of the Soay sheep genome influence FEC (Coltman *et al*., 2001b; Beraldi *et al*., 2007).

Previous work in this population has also shown that individual FEC is correlated with host body size at both the phenotypic and genetic level (Coltman *et al*., 2001a; Robinson *et al*., 2009), and is associated with variation in individual behaviour (Hutchings *et al*., 2002), host sex (Wilson *et al*., 2004), and host age (Craig *et al*., 2008; Hayward *et al*., 2009). Research in domesticated populations suggests that sheep immunity regulates FEC via effects on both worm number and fecundity (Smith *et al*., 1985; Stear *et al*., 1995), and that a large proportion of the variance in FEC is due to host genotype (Stear *et al*., 1997; Stear *et al*., 2009). This high degree of individual-based variation in FEC demonstrates its usefulness as an inverse measure of parasite resistance, as is widespread in the veterinary parasitology literature where FEC is the target for selection for enhanced parasite resistance (Woolaston, 1992; Gruner *et al*., 2004a; Sayers & Sweeney 2005).

Experimental studies in domesticated sheep have relieved parasite burden using anthelmintic drugs, and have shown that treatment can increase survival and reproductive performance

(Gatongi *et al*., 1997; Thomson *et al*., 2000). Similar experiments on St Kilda have shown that treated lambs and yearlings have higher survival (Gulland, 1992; Gulland *et al*., 1993), but that treatment has no effect on adult survival (Craig *et al*., 2009). There is also some experimental evidence that gastro-intestinal nematodes may affect female reproduction in a complex way (Tempest, 2005). Individual FEC is negatively correlated with host body weight in this population, which is itself under positive selection (Milner *et al*., 1999a; Pelletier *et al*., 2007). This has led to speculation that apparent selection on weight may be due to correlated selection on parasite resistance (Milner *et al*., 1999a). However, despite extensive longitudinal data, the associations between naturally-occurring FEC and individual fitness are unknown. Furthermore, the genetic correlation between FEC and body weight changes across ages and environmental conditions (Robinson *et al*., 2009), and the strength of selection on body size also varies between years (Milner *et al*., 1999a; Pelletier *et al*., 2007). These observations suggest that patterns of selection on FEC, and the implications of associated selection on body weight, may vary across different age groups and across years.

Using individual FEC as a measure of parasite resistance, and considering associations between fitness and FEC, we aim to elucidate the patterns of selection acting on parasite resistance. We performed selection analysis using multiple regressions of standardized trait values on a measure of annual fitness which incorporates survival and reproduction. We tested for linear and non-linear associations between FEC and annual fitness. We then tested for selection on the correlated trait of body weight, and for correlational selection on FEC and body weight (Lande & Arnold, 1983; Mitchell-Olds & Shaw, 1987). Finally, we attempted to quantify the extent of variation in selection between years of high and low mortality, and also tested for age-related changes in the form of selection acting on FEC. We predict that selection on FEC should be stronger in lambs, since these have the highest parasite infection intensities (Craig *et al*., 2009)

and lowest annual survival rates (Clutton-Brock *et al*., 1991). We also predict that FEC will covary with fitness more strongly in adverse environmental conditions. Our findings should aid understanding of some of the factors contributing to the complexity of selection on parasite resistance in natural populations.

5.3 Methods

5.3.1 Study population and data collection

The free-living population of Soay sheep on the island of Hirta, in the St Kilda archipelago, NW Scotland (57°49′N 08°34′W), has been the subject of a longitudinal individual-based study since 1985 (Clutton-Brock & Pemberton, 2004). The size of the population in the intensively-studied Village Bay area (around a third of the island) fluctuates between 200 and 700 individuals, with occasional large declines in population size due to high winter mortality, known as crashes (Figure 5.1). High mortality is associated with high population density, adverse weather, and low food availability (Coulson *et al*., 2001), and in high mortality years survivors lose more weight than in low mortality years (Clutton-Brock *et al*., 1992). Crashes are therefore considered to represent adverse environmental conditions where more individuals die and survivors experience lower condition than at other times (Clutton-Brock *et al*., 1992). Lambs are born in April, and 95% of the Village Bay population are caught, weighed, blood- and tissue-sampled, and given individual identification tags within a week of birth (Clutton-Brock & Pemberton, 2004). Maternities are assigned on the basis of behavioural interactions between lambs and ewes, and paternity is assigned from a pedigree constructed using the R package MasterBayes (Hadfield *et al*., 2006). Lambs are suckled until weaning around June, and are independent by August

(Clutton-Brock *et al*., 2004a). Each August, over 50% of the population are caught and weighed (to the nearest 0.1kg), measured, bled, and faecal samples are taken in which parasite eggs (see Introduction) are counted using the McMaster egg counting technique (M.A.F.F., 1986). Mortality in the population is chiefly due to protein malnutrition (Gulland, 1992), and largely occurs over the winter from January to May. Mortality is biased towards juveniles and males, but can also be heavy in adult females during crash winters (Clutton-Brock *et al*., 1991; 1992).

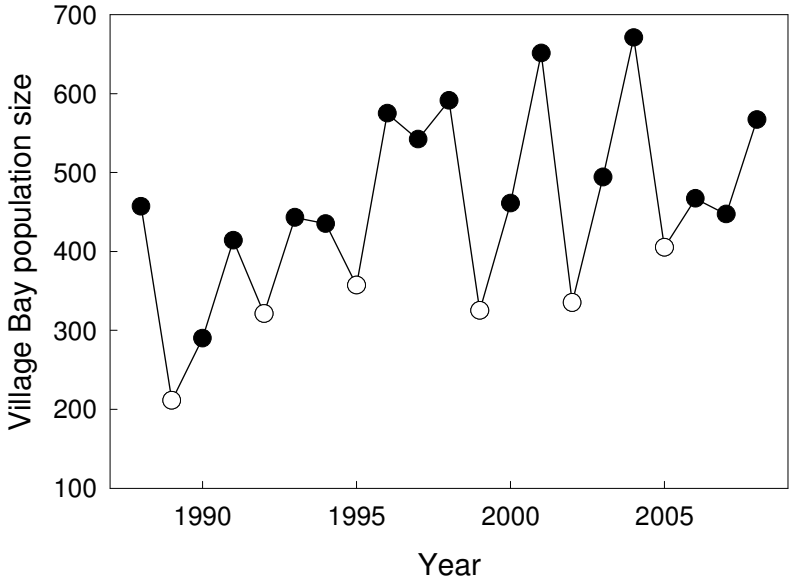

Figure 5.1: The Village Bay study population on Hirta shows dramatic fluctuations in population size. Every few years, a combination of high population density, poor weather, low food availability and high parasite burdens cause a sharp decline in population size, known as 'crashes'. Points show population size estimated in October of the year indicated, with filled symbols indicating that the previous winter was not a crash, and open symbols showing years where the preceding winter was a crash.

5.3.2 Selection analyses

We performed selection analyses considering associations between an annual measure of fitness and FEC using multiple regression of fitness on standardized traits to estimate standardized

selection gradients (Lande & Arnold, 1983). We analysed 2419 samples collected from 1350 individuals of known age and weight from 1988-2008. The partial regression coefficients from these analyses are a measure of the strength of selection on each trait (Price *et al*., 1984), and have been widely used in evolutionary ecology (Kingsolver *et al*., 2001). This method estimates selection from the covariance between a phenotypic trait and a measure of fitness, and makes no assumption about underlying genetic variance of the trait. Using this technique, causality is often assumed, with the caveat that the observed association between a trait and fitness may arise from an unknown (and unmeasured) trait or factor; measuring likely sources of variation in fitness and including them in analyses is one way of validating this assumption (Lande & Arnold, 1983; Mitchell-Olds & Shaw, 1987. From herein, we use the term 'selection' to describe a phenotypic association between a given trait and a measure of fitness.

Lifetime fitness may be defined as the total number of progeny contributed by an individual to the next generation. However, natural selection is a continuous process which acts on individuals at a given point in time and is not concerned with per generation fitness, and so considering selection across time intervals shorter than generations may be informative (Coulson *et al*., 2006). Many studies have assessed selection gradients between a phenotypic trait and survival or fecundity (Kingsolver *et al*., 2001), but selection acts on both of these, and so selection estimated using only one of these traits will not necessarily be informative in predicting evolutionary change (Lande, 1982). In iteroparous animals, lifetime fitness is the sum of the contributions of many reproductive events across discrete time periods, but understanding selection on age-dependent traits and traits which change across environmental conditions requires investigation of the association between the trait and reproductive success within that period, plus survival to the next time period, i.e. the annual contribution to lifetime fitness (e.g. Gustafsson, 1987; Brommer *et al*., 2007; Cockburn *et al*., 2008; Engen *et al*., 2009; Wilson *et*

al., 2009). This represents the genetic contribution of an individual to the next time step, and allows incorporation of the fitness components survival and reproduction into a single variable, as well as analysis of age- and environment-specific variation in selection. Using the models described below, our selection gradients therefore describe the associations between annual contribution to lifetime fitness in a given year and observed FEC. With FEC and body weight (WT) measured in August of year *t*-1, we defined annual contribution to lifetime fitness *W* of individual *i* over the following winter and breeding season *t*, as:

$$W_{i,t} = p_{i,t} + (0.5 \text{ x } r_{i,t})$$

where $p_{i,t}$ is the survival of individual *i* over the winter to May 15th of year *t*, and $r_{i,t}$ is the reproductive output of individual *i* at time *t* (following Brommer *et al*., 2007). For brevity, we refer to annual contribution to lifetime fitness simply as 'annual fitness'. Throughout, we performed separate analysis for either sex. This allowed us to define reproductive output $r_{i,t}$ slightly differently for either sex: for males, $r_{i,t}$ is the number of lambs sired that were born in the spring of year *t*; for females, $r_{i,t}$ is the number of lambs born in year *t* and surviving to the August of that year, thus incorporating a measure of juvenile survival into our measure of female reproductive output, since early offspring survival is strongly linked to various aspects of maternal phenotype (Jones et al., 2005).

Across age and sex classes, there is substantial variation in the distributions of individual mortality rate, reproductive performance, body weight, and parasite burden (Clutton-Brock *et al*., 1991; 1992; 2004a; Craig *et al*., 2006). For instance, lambs may experience substantially higher FEC than both yearlings and adults (Craig *et al*., 2006), and males aged 2 and over achieve far higher reproductive success than those aged under 2 (Robinson *et al*., 2006). For this

reason, we split our data into six age- and sex- specific groups: female and male lambs (around four months of age at faecal sampling), female and male yearlings (around 16 months of age), and female and male adults (28 months of age or over), potentially for multiple years across their lifetimes. We then divided absolute annual fitness $W_{i,t}$ by the mean within each class to give relative annual fitness $w_{i,t}$ for each year of life for each individual.

5.3.2.1 Selection on FEC and body weight

We built up a series of selection analyses testing for associations between annual fitness and FEC alone, then a multivariate analysis with FEC and WT, and finally models incorporating age and environment-specific variation.

Our longitudinal data include many repeated counts from individuals over many years, so we used linear mixed-effects models (LMMs) to analyse selection via relative annual fitness, with individual identity and year as random effects in analyses of adults, and with year as a random effect in analyses of lambs and yearlings. We initially estimated selection on FEC using a univariate model, and under the implicit assumption that the relationship between FEC and fitness is independent of other phenotypic traits, age or environmental conditions. FEC + 1 was natural log-transformed due to its highly overdispersed distribution; Ln(FEC+1) and WT were standardized to zero mean and unit S.D. within each age and sex subset in order to estimate standardized selection gradients. We tested for linear and non-linear selection with linear and quadratic terms for standardized FEC. Model 1a was used to analyse lambs and yearlings; model 1b was used to analyse adults:

$w_{i,t} \sim \text{FEC} + \text{FEC}^2 + \text{year}_t$ (model 1a)

$w_{i,t} \sim \text{FEC} + \text{FEC}^2 + \text{identity}_i + \text{year}_t$ (model 1b)

By including identity_i and year_t as random effects, we accounted for non-independence of multiple records from the same individual, and for unexplained environmental variance at both the individual and population level. We next considered the extent of correlated selection with weight by including linear and quadratic terms for standardized WT, and the interaction between FEC and WT (model 2a and 2b for lambs/yearlings and adults respectively).

$w_{i,t} \sim \text{WT} + \text{WT}^2 + \text{FEC} + \text{FEC}^2 + \text{WT.FEC} + \text{year}_t$ (model 2a)

$w_{i,t} \sim \text{WT} + \text{WT}^2 + \text{FEC} + \text{FEC}^2 + \text{WT.FEC} + \text{identity}_i + \text{year}_t$ (model 2b)

This model ascertained whether any selection on FEC found in model 1 could be explained by correlated selection on body weight (Lande & Arnold, 1983; Phillips & Arnold, 1989), and the interaction term tested for correlational selection between FEC and WT. Regression analysis of standardized FEC on standardized WT showed the two to be significantly negatively correlated in all age and sex subsets apart from adult males (Figure 5.2).

5.3.2.2 Age- and environment- related variation in selection

We next expanded model 2 to relax the assumption that selection on FEC and WT are constant across environmental conditions and ages. In model 3, we tested whether relative fitness differed

between crash years and non-crash years (as indicated in Figure 5.1), and whether selection on FEC and WT was dependent on whether it was a crash year or not. Model 3 was used to analyse lambs and yearlings of each sex separately:

$$w_{i,t} \sim \text{CRASH} + \text{WT} + \text{WT}^2 + \text{FEC} + \text{FEC}^2 + \text{WT.FEC} + \text{CRASH.WT} + \text{CRASH.WT}^2 + \text{CRASH.FEC} + \text{CRASH.FEC}^2 + \text{CRASH.WT.FEC} + \text{year}_t \quad \text{(model 3)}$$

where CRASH is a two-level factor and all other terms are as described above. We then analysed adults using a model which allowed annual fitness to change with age, the effects of crash years to vary across ages, and selection on FEC and WT to change across ages and between crash and non-crash years. Age was standardized to zero mean and unit variance, and included in model 4:

$$w_{i,t} \sim \text{age} + \text{age}^2 + \text{CRASH} + \text{WT} + \text{WT}^2 + \text{FEC} + \text{FEC}^2 + \text{WT.FEC} + \text{age.CRASH} + \text{age.WT} + \text{age.FEC} + \text{CRASH.WT} + \text{CRASH.FEC} + \text{identity}_i + \text{year}_t \quad \text{(model 4)}$$

We also ran a model with three-way interactions between age, FEC and WT, and between CRASH, FEC, and WT, as well as interactions between CRASH and quadratic WT and FEC, and between age and quadratic WT and FEC. These interactions were always non-significant and added complexity to the model which was of questionable value with sample sizes of only 150 in some age and sex subsets, and so we exclude these interactions from the presented analysis for simplicity.

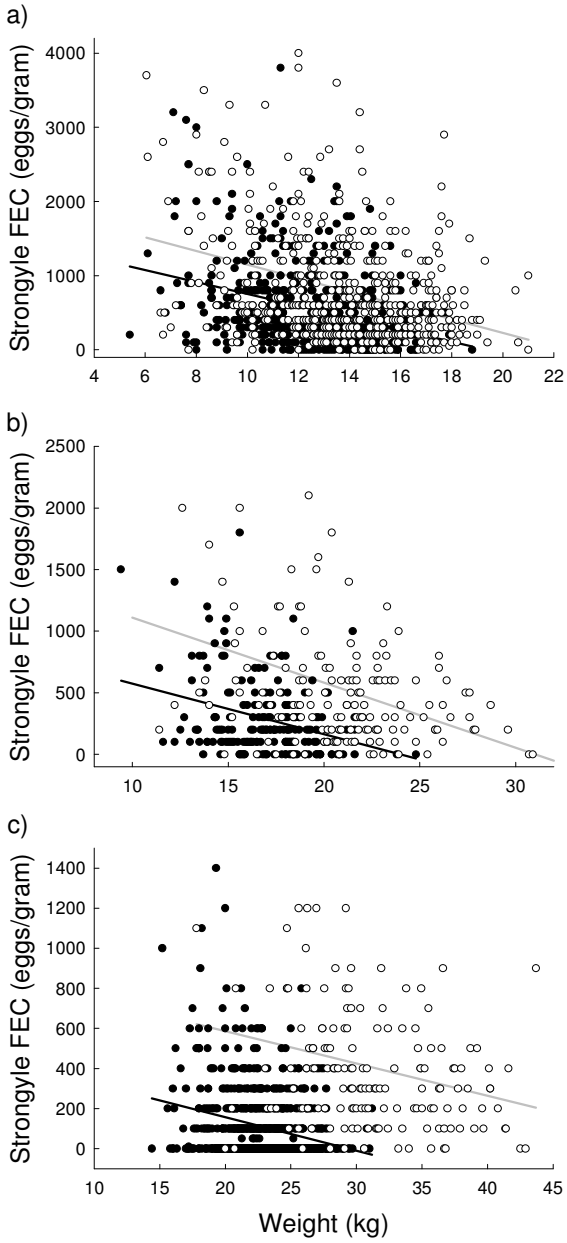

Figure 5.2: FEC is negatively correlated with body weight in a) lambs, b) yearlings, and c) adults. Filled symbols show female data and the black lines show regression fitted to the raw data; open symbols show male data and grey lines show the fitted regression. Linear regression of standardized FEC on standardized WT showed that the negative relationship was significant in all sex and age subsets apart from adult males; parameter estimates ± 1S.E. for effects of standardized WT on standardized FEC: Female lambs = -0.2956 ± 0.0413, p = <0.001; Male lambs = -0.2247 ± 0.0428, p = <0.001; Female yearlings = -0.2847 ± 0.0643, p = <0.001; Male yearlings = -0.1890 ± 0.0783, p = 0.017; Female adults = -0.1801 ± 0.0335, p = <0.001; Male adults = -0.1309 ± 0.0782, p = 0.096. Quadratic terms were tested but all were non-significant.

All models were simplified by sequentially dropping non-significant fixed effects from the full model, with significance assessed from Wald F-statistics, distributed as χ^2 on the appropriate degrees of freedom, with $\alpha = 0.05$. In presenting results, we report statistics associated with dropping the term in question from the model. All analysis was performed using the GLMM procedure with normal errors in GenStat 12th Edition (VSN International, Hemel Hempstead, U.K.); we report parameter estimates for fixed effects ± 1 S.E.

5.4 Results

5.4.1. Associations between FEC and annual fitness

The results of models 1a and 1b suggested that annual contribution to lifetime fitness was negatively associated with FEC. There was a significant negative linear association between annual fitness and FEC in both female lambs (estimate = -0.2096 ± 0.0417, $Wald_{1,578} = 25.31$, p = <0.001) and male lambs (FEC = -0.2917 ± 0.0855, $Wald_{1,517} = 11.64$, p = <0.001). In males there was also evidence for a non-linear (quadratic) component to the selection (FEC^2 = -0.0659 ± 0.0264, $Wald_{1,513} = 0.80$, p = 0.013); the negative parameter estimate for the quadratic suggested that annual fitness decreased with FEC more rapidly as FEC increased, and that therefore there was no evidence for stabilizing or disruptive selection. In yearlings there was no significant selection on FEC in females (FEC = -0.0315 ± 0.0353, $Wald_{1,210} = 0.80$, p = 0.373), but linear and non-linear effects were again significant in males (FEC = -0.3071 ± 0.1133, $Wald_{1,149} = 7.34$, p = 0.008; FEC^2 = -0.1549 ± 0.0482, $Wald_{1,147} = 10.35$, p = 0.002), again predicting a convex negative curve and that the negative association between annual fitness and FEC accelerated as FEC increased. Finally, the linear effect was weak but significant in adult

females (FEC = -0.0355 ± 0.0142, $Wald_{1,895}$ = 6.27, p = 0.012), but not significant in adult males (FEC = 0.0425 ± 0.0795, $Wald_{1,157}$ = 0.29, p = 0.594). The quadratic terms were not significant for adults of either sex.

Next we performed a bivariate selection analysis on FEC and WT as correlated traits, using models 2a and 2b. In female lambs, there was significant positive association between annual fitness and WT, which predicted a positive concave relationship, but even having accounted for this we still found a significant linear negative association with FEC (Table 5.1a). There was also a positive linear association between annual fitness and WT in male lambs, and a significant negative linear association with FEC (Table 5.2a). The interaction term testing for correlational selection was not significant in either sex (female estimate = 0.0532 ± 0.0429, $Wald_{1,512}$ = 1.53, p = 0.216; male estimate = -0.0460 ± 0.0511, $Wald_{1,475}$ = 0.81, p = 0.369).

In female yearlings, there was a significant positive linear association between annual fitness and WT (Table 5.1b), but no significant selection on FEC (0.0075 ± 0.0364, $Wald_{1,189}$ = 0.04, p = 0.838). In males, having accounted for a significant positive association between annual fitness and WT (Table 5.2b), there was no significant association with FEC (0.0411 ± 0.0587, $Wald_{1,145}$ = 0.49, p = 0.486). In female adults, annual fitness was significantly positively associated with WT (Table 5.1c), and after accounting for this there was no significant selection on FEC (-0.0132 ± 0.0147, $Wald_{1,835}$ = 0.80, p = 0.371). In male adults, annual fitness was not significantly associated with FEC (0.0513 ± 0.0740, $Wald_{1,145}$ = 0.48, p = 0.489), but was strongly and positively associated with WT (Table 5.2c).

	Variables	Estimate	S.E.	Wald	d.f.	p
a)	***Lambs***					
	Fixed effects					
	Intercept	1.0340	0.1836			
	WT	0.2669	0.0437	37.36	1	<0.001
	WT^2	0.0668	0.0298	5.02	1	0.025
	FEC	-0.1287	0.0434	8.80	1	0.003
	Variance components					
	Year	0.6565	0.2218			
	Residual	0.7700	0.0482			
b)	***Yearlings***					
	Fixed effects					
	Intercept	0.9615	0.0977			
	WT	0.1977	0.0341	33.57	1	<0.001
	Variance components					
	Year	0.1376	0.0575			
	Residual	0.1830	0.0191			
c)	***Adults***					
	Fixed effects					
	Intercept	0.9530	0.0560			
	WT	0.0677	0.0151	20.09	1	<0.001
	Variance components					
	ID	0.0047	0.0054			
	Year	0.0575	0.0215			
	Residual	0.1650	0.0095			

Table 5.1: Results from model 2a for female lambs (534 samples from 534 individuals) and yearlings (201 samples from 201 individuals), and from model 2b for female adults (861 samples from 296 individuals). Estimates and SE are from final LMMs analysing associations between annual fitness, FEC and WT.

	Variables	Estimate	S.E.	Wald	d.f.	p
a)	***Lambs***					
	Fixed effects					
	Intercept	1.2260	0.2602			
	WT	0.1725	0.0545	10.02	1	0.002
	FEC	-0.1309	0.0548	5.71	1	0.017
	Variance components					
	Year	1.3290	0.0770			
	Residual	1.1860	0.0770			
b)	***Yearlings***					
	Fixed effects					
	Intercept	0.9717	0.1677			
	WT	0.2337	0.0584	16.00	1	<0.001
	WT^2	-0.1013	0.0353	8.23	1	0.005
	Variance components					
	Year	0.3756	0.1616			
	Residual	0.4220	0.0505			
c)	***Adults***					
	Fixed effects					
	Intercept	1.1190	0.1731			
	WT	0.5078	0.0805	39.82	1	<0.001
	WT^2	0.1906	0.0609	9.81	1	0.002
	Variance components					
	ID	0.1272	0.0935			
	Year	0.4100	0.1903			
	Residual	0.5900	0.1030			

Table 5.2: Results from model 2a for male lambs (497 samples from 497 individuals) and yearlings (157 samples from 157 individuals), and from model 2b for male adults (154 samples from 102 individuals). Estimates and S.E. are from final LMMs analysing associations between annual fitness, FEC and WT.

5.4.2 Selection across ages and crash years

In all age- and sex-specific data subsets, annual fitness was significantly lower in crash years than in non-crash years. Contrary to our *a priori* prediction, model 3 showed that the negative association between annual fitness and FEC was stronger in non-crash years than in crash years (Table 5.3), suggesting that there was negligible selection on FEC in crash years, but that there was negative selection on FEC in non-crash years (Figure 5.3). Annual fitness was positively

associated with WT in both sexes, and this relationship did not differ significantly between crash and non-crash years. In yearlings, only WT and crash year were significantly associated with annual fitness, with a positive effect of WT (Female = 0.2001 ± 0.0331, $Wald_{1,196} = 36.52$, $p < 0.001$; Male WT = 0.2293 ± 0.0580, $Wald_{1,152} = 15.64$, $p < 0.001$; Male $WT^2 = -0.0870 \pm 0.0353$, $Wald_{1,151} = 6.08$, $p = 0.015$), and lower fitness in crashes (Female = -0.6299 ± 0.1105, $Wald_{1,12} = 32.49$, $p < 0.001$; Male = -0.8254 ± 0.2637, $Wald_{1,12} = 9.80$, $p = 0.009$).

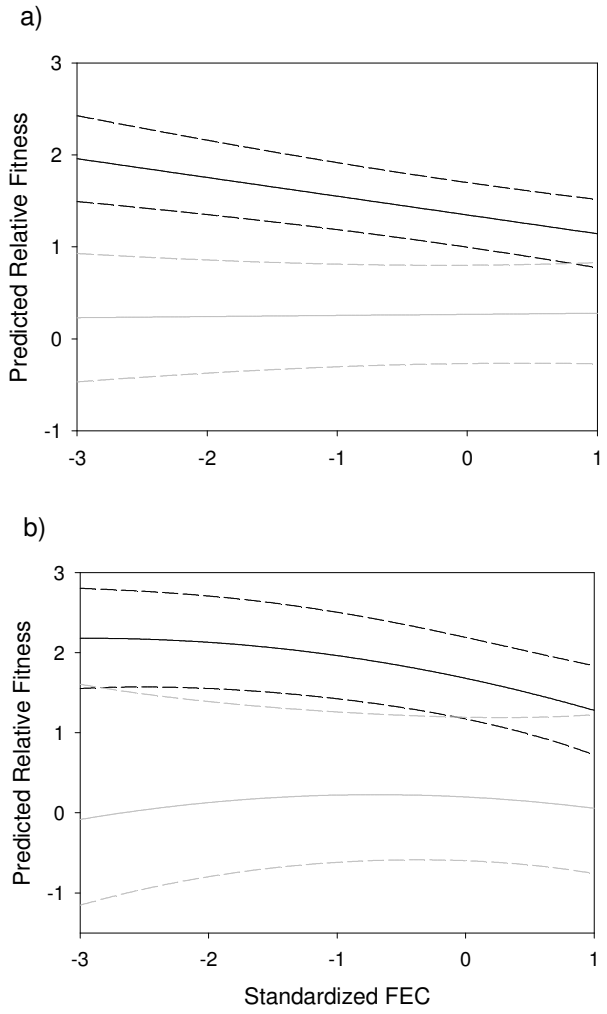

Figure 5.3: Results from model 3, showing predicted relative annual fitness as a function of standardized faecal egg count for a) female lambs and b) males lambs. In both case, FEC is negatively associated with relative annual fitness in non-crash years (black lines), but is not associated with fitness in crash years (grey lines). Solid lines indicate model predictions; dashed lines indicate 95% confidence intervals.

	Variables		Estimate	S.E.	Wald	d.f	p
a)	***Females***						
	Fixed effects						
	Intercept		1.3470	0.1759			
	WT		0.2684	0.0434	38.22	1	<0.001
	WT^2		0.0680	0.0297	5.26	1	0.022
	FEC		-0.2039	0.0531	9.54	1	0.002
	Crash						
		0	0.0000		11.26	1	0.004
		1	-1.0807	0.3201			
	FEC x Crash						
		0	0.0000		5.97	1	0.015
		1	0.2162	0.0885			
	Variance components						
	Year		0.4048	0.1471			
	Residual		0.7640	0.0479			
b)	***Males***						
	Fixed effects						
	Intercept		1.6210	0.2540			
	WT		0.1593	0.0548	8.46	1	0.004
	FEC		-0.3420	0.1024	5.63	1	0.018
	FEC^2		-0.0587	0.0259	4.22	1	0.041
	Crash						
		0	0.0000		10.81	1	<0.001
		1	-1.4794	0.4711			
	FEC x Crash						
		0	0.0000		4.02	1	0.046
		1	0.2596	0.1295			
	Variance components						
	Year		0.8700	0.3140			
	Residual		1.1770	0.0770			

Table 5.3: Results of model 3 (LMM analyzing selection on body weight and FEC, effects of crash years, and differences in selection between crash years) via relative annual fitness in a) female lambs and b) male lambs.

For adult females, the results of model 4 provided no support for significant selection on FEC (-0.0058 ± 0.0139, Wald = 0.18, d.f. = 1, p = 0.675), after conditioning on WT and age-related sources of variation (Table 5.4). We found that annual contribution to lifetime fitness was significantly lower in crash years, and peaked around age 5, with the decline in annual fitness in the oldest sheep steeper in crash years than in non-crash years (Figure 5.4a). Selection on WT strengthened with increasing age within the adult class, with apparently weak selection in young and prime-aged adult females but strong positive selection in the oldest females (Figure 5.5). Finally, selection on WT was stronger in crash years (Figure 5.4b).

Variables		**Estimate**	**S.E.**	**Wald**	**d.f.**	**p**
Fixed effects						
Intercept		1.0840	0.0272			
WT		0.0671	0.0196	35.16	1	<0.001
Crash						
	0	0.0000		61.87	1	<0.001
	1	-0.3477	0.0449			
Age		-0.0220	0.0072	101.07	1	<0.001
Age^2		-0.0116	0.0019	38.03	1	<0.001
Crash x Age						
	0	0.0000		13.22	1	<0.001
	1	-0.0398	0.0110			
WT x Age		0.0178	0.0049	13.28	1	<0.001
Crash x Age						
	0	0.0000		8.92	1	0.003
	1	0.0867	0.0290			
Variance components						
ID		0.0174	0.0063			
Year		0.0048	0.0033			
Residual		0.1350	0.0080			

Table 5.4: Final result of model 4 (LMM analyzing selection on body weight and FEC, effects of age and crash years, and changes in selection across ages and crash years) via relative annual fitness in female adults aged 2-12 (861 samples from 295 individuals). Five measures from 13-year-olds and one measure from a 14-year-old were included as age 12.

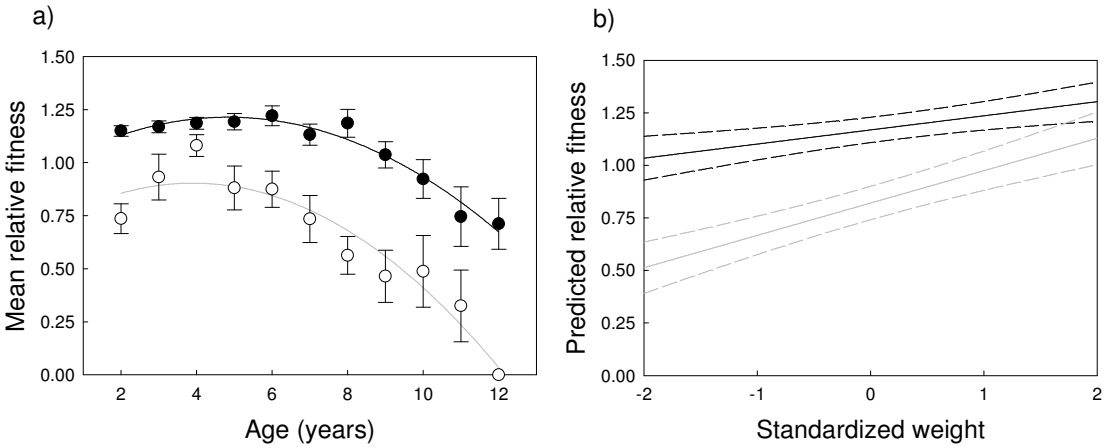

Figure 5.4: The results of model 4 for adult females showed that a) relative annual fitness declines more sharply with age in crash years (open symbols, grey line) than it does in non-crash years (filled symbols, black line). A decline in fitness with age is apparent from the age of 6 in crash years, but not until the age of 8 in non-crash years. Points show mean relative fitness at each age ± 1 S.E.; lines show best fit through the plotted data. Results also show that b) selection on body weight in crash years (grey) is predicted to be stronger than in non-crash years (black). Solid lines show predictions derived from parameter estimates of model 4; dashed lines indicate 95% confidence intervals.

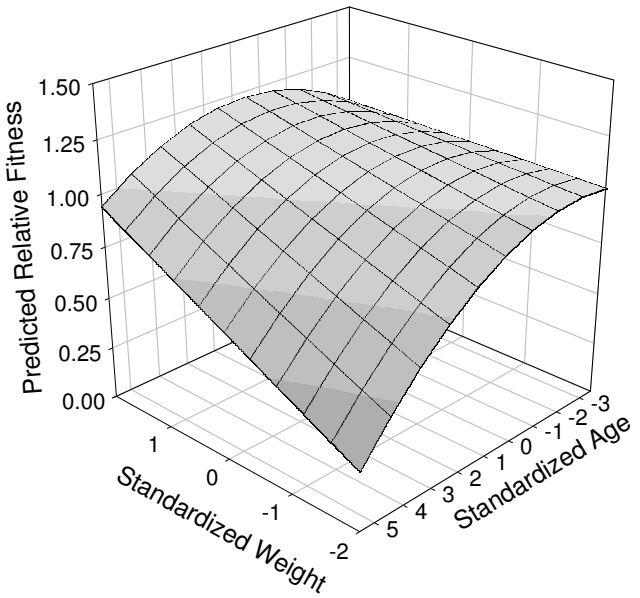

Figure 5.5: Surface plot of predictions from model 4 (Table 5.4) for adult females. Results suggest that selection on body weight is negligible at early ages, but becomes increasingly positive at older ages.

In adult males, we found that annual contribution to lifetime fitness was significantly and positively associated with WT, and that males had lower annual fitness in crash years (Table 5.5). The significant interaction between WT and age indicates that positive selection was strongest in the oldest individuals (Figure 5.6a). FEC was not significantly associated with annual fitness overall, but the significant interaction with age suggested that there was no association in young and middle-aged males, but a positive association in the oldest males, a surprising indicator of positive selection on FEC in these individuals (Figure 5.6b). The significant interaction between FEC and WT suggested that there was a tendency for a negative association between FEC and relative fitness in heavy males, but a positive tendency in light males.

Variables		**Estimate**	**S.E.**	**Wald**	**d.f.**	**p**
Fixed effects						
Intercept		1.3120	0.1996			
WT		0.3593	0.1098	39.69	1	<0.001
FEC		-0.0150	0.0732	0.18	1	0.668
Crash						
	0	0.0000		6.62	1	0.025
	1	-0.7964	0.3096			
Age		0.0634	0.0949	2.02	1	0.157
WT x FEC		-0.1519	0.0669	5.16	1	0.025
WT x Age		0.1861	0.0733	6.45	1	0.012
FEC x Age		0.1764	0.0610	8.37	1	0.004
Variance components						
ID		0.1611	0.0964			
Year		0.2918	0.1567			
Residual		0.5400	0.0985			

Table 5.5: Final result of model 4 (analyzing selection on body weight and FEC, effects of age and crash years, and changes in selection across ages and crash years) via relative annual fitness in male adults aged 2-6 (154 samples from 102 individuals). One measure from a seven-year-old and one measure from an eight-year-old were included as age 6.

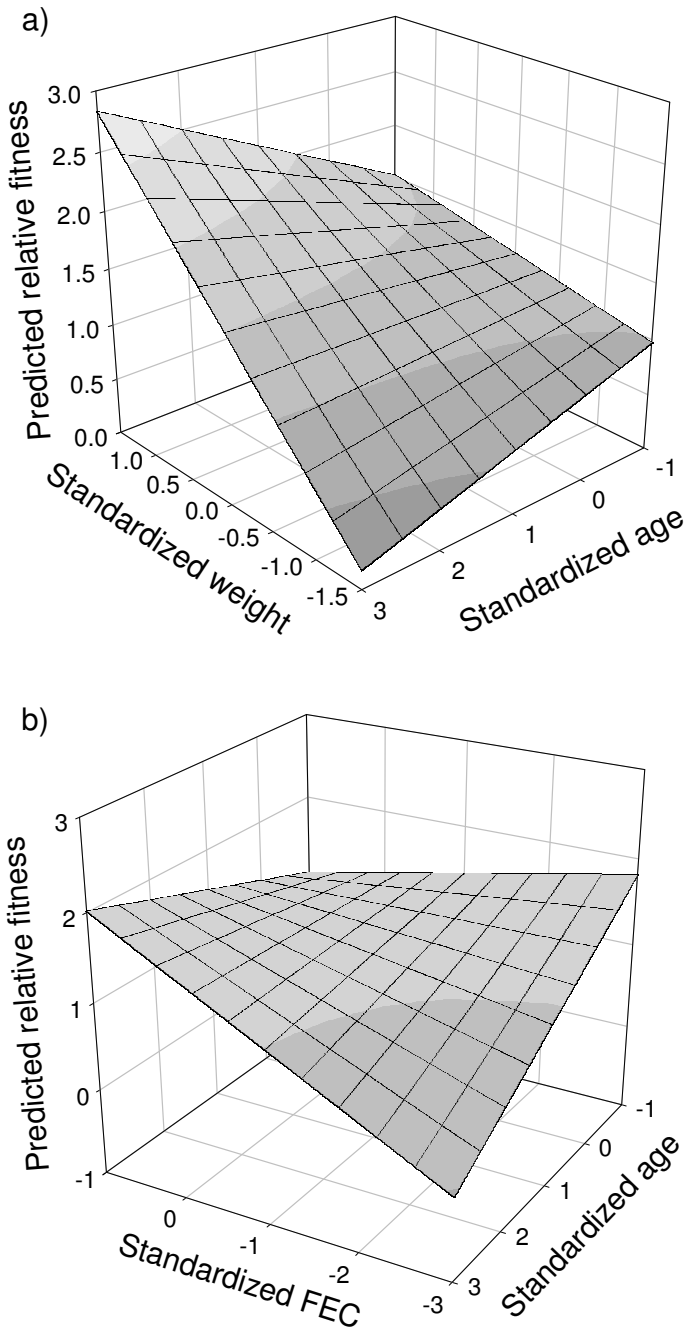

Figure 5.6: Surface plots of predictions from model 4 (Table 5) for adult males. Results suggest that a) as in adult females, selection on body weight strengthens with increasing age; b) although there is no selection on FEC overall, there is a suggestion that increasing FEC is positively associated with annual fitness in the oldest males.

5.5 Discussion

In this study, we have performed selection analysis on natural variation in a measure of parasite resistance in a longitudinally-monitored mammal population. Our results suggest that, once covariance with weight is accounted for, there is positive selection on parasite resistance (i.e. negative associations between fitness and FEC) in lambs of both sexes but not in older sheep. We also found that the magnitude of selection on both FEC and weight changed across ages and between crash and non-crash years for particular age and sex classes. This study is a rare attempt to quantify selection on a measure of parasite resistance in a natural population, and shows the importance of age and environmental conditions in determining selection regimes.

5.5.1 Selection on FEC via annual fitness

We found a significant negative association between a measure of annual fitness and FEC in lambs, yearling males, and adult females. However, once we accounted for effects of body weight on fitness, the association between FEC and fitness was only significant in lambs, emphasizing the importance of accounting for correlated characters in selection analysis (Lande & Arnold, 1983; Phillips & Arnold, 1989). FEC is treated as a measure of parasite resistance in this population and in domesticated sheep populations under the assumptions outlined in the Introduction. Assuming that FEC reflects individual parasite resistance, the results suggested that there was phenotypic selection for enhanced parasite resistance in lambs, but that in older animals, selection on parasite resistance was either absent, or indirect and occurring through positive selection on the correlated trait of body weight. Experimental studies in this population have shown that anthelmintic treatment improves survival in lambs and yearlings (Gulland,

1992; Gulland *et al*., 1993), but not adults (Craig *et al*., 2009). Our results therefore support experimental data to some extent, but also reveal more details of the relationships between FEC and host fitness. The prevalence of strongyles in lambs is 95%, and so very few lambs have zero FEC under natural conditions (Craig *et al*., 2008) and therefore experiments may not have been representative of the natural distribution of FEC. We were able to test for non-linear selection on FEC, although we found no evidence for stabilizing selection on FEC and therefore no significant cost to parasite resistance. Thus, our results suggest that the association between FEC and fitness in lambs is linear across the full range of natural variation in FEC, with individuals with very high FEC having the lowest fitness, something which experimental studies could not test. Possible reasons for our not finding significant selection on FEC in yearlings may be related to the fact that we conditioned our analyses on WT, and that the experiment of Gulland *et al*. (1993) was conducted over a single crash winter. In such a winter, individuals relieved of parasite pressure were compared with individuals which were likely to have high levels of parasites due to the high population density and their poor condition.

The current findings and those presented previously (Gulland, 1992; Gulland *et al*., 1993; Craig *et al*., 2009) raise the question of why FEC should be negatively associated with annual fitness in lambs but not in adults. Mean August FEC is around four times higher in lambs (Craig *et al*., 2008), and the relative frequencies of different species of strongyles change across ages; in lambs that die over winter, over 80% of mature worms are *Trichostrongylus* spp., while these species account for only around 50% of mature worms in adult sheep, which is associated with a large decline in absolute numbers of *Trichostrongylus* spp. (Craig *et al*., 2006). In domesticated sheep, this age-related decrease in *Trichostrongylus* spp. is due to the development of immunity as sheep mature (Waller & Thomas, 1981; Smith *et al*., 1985). A possible target of selection is therefore development of resistance to *Trichostrongylus* spp. over the first summer, since

Trichostrongylus vitrinus is the worm species most closely associated with over-winter weight loss (Craig *et al*., 2009). Alternatively, if FEC reflects exposure to parasite larvae rather than resistance, it may be lambs that are exposed to more parasites that have higher fitness. Support for the resistance hypothesis comes from an experimental study which infected domesticated Soay lambs with a fixed dose of *T. circumcincta* larvae, and showed that males harboured more worms at slaughter than did females. This suggests individual differences in the rate of establishment of parasites due to host sex (Gulland, 1991), and raises the possibility that other sources of individual heterogeneity could contribute to variation in parasite resistance. Hence, lambs that develop effective resistance to *Trichostrongylus* spp. could experience higher fitness, and selection for higher parasite resistance over the first winter of life could be a reason for the lower prevalence of *Trichostrongylus* spp. in those that survive to adulthood.

Despite no evidence for significant selection on FEC in adults, there was at least a qualitative suggestion that the relationship between FEC and fitness is actually positive in the oldest adult males (Figure 5.5b). It is possible that the oldest males may adopt a strategy of terminal investment, in which, in the face of physiological deterioration, increased parasite load, and declining survival prospects, they invest heavily in reproduction (e.g. Bonneaud *et al*., 2004; Velando *et al*., 2006) and less in immunity and so achieve higher annual fitness despite having higher FEC. However, only 22 samples were obtained from a total of 16 males aged 5 and over, and the data set for adult males was substantially smaller than that for lambs and adult females due to lower male lifespan. It is possible that the result is a type I error (a false positive); even if not, the biological significance of selection at this age, in terms of its potential impact on life-history evolution or population dynamics, is questionable.

5.5.2 Selection on FEC across environmental conditions

In lambs of both sexes, selection on FEC was negative in non-crash years, and absent in crash years. Assuming that crash years represent harsher environmental conditions (Clutton-Brock *et al*., 1992), this is contrary to the general expectation that selection should be stronger in more adverse environmental conditions (e.g. Verhulst, 1998; Wilson *et al*., 2006). Thus, we have found evidence for variation in selection across environmental conditions, but in a direction contrary to that expected from evolutionary theory and previous empirical evidence. A simple explanation for this is that mortality in lambs in crash years is so high (72-95%) that there is little variance in fitness and so little opportunity for selection. However, there is positive selection on body weight in lambs during both crash and non-crash years (Table 5.3), suggesting that this explanation is not sufficient. It may be that under such conditions, FEC is generally high in the population and becomes uncoupled from body weight, which is under stronger selection during these winters. Comparison with estimates of selection gradients on other phenotypic traits in other populations, suggests that in crash years selection on FEC in lambs is relatively strong (Kingsolver *et al*., 2001).

As discussed above, there is considerable genetic variance for FEC in this population (Coltman *et al*., 2001a; Beraldi *et al*., 2007), and given the evidence we have presented here for negative selection on FEC, we might ask what maintains this variance. The potential mechanisms underlying the maintenance of variation in FEC include temporal and spatial environmental heterogeneity and variation in worm genetics, neither of which are under the control of individual sheep. However, a large number of individual-level differences do contribute to variation in FEC, including the genetic complexity of immunity (Paterson *et al*., 1998; Beraldi

et al., 2007), challenge by a wide range of parasites eliciting different types of immune responses (Wilson *et al*., 2004; Cox, 2001), and the fact that parasite resistance is likely to be condition-dependent. Individuals with greater resources at their disposal are likely to have higher parasite resistance, and since condition is a complex trait determined by many genetic and non-genetic factors it may show substantial genetic variance (Price & Schluter, 1991; Rowe & Houle, 1996), leading to the expectation of considerable variation in FEC. These factors are non-mutually exclusive, and our results here provide evidence that variable selection across ages and years could be another factor maintaining variation in parasite resistance in natural populations.

5.5.3 Selection on body weight

Previous work has shown that body weight is positively associated with both survival (Clutton-Brock *et al*., 1992; Jones *et al*., 2005) and reproductive success (Clutton-Brock *et al*., 1996; Coltman *et al*., 1999b) in the study population. Our study has confirmed this, and has also shown variation in selection on weight across ages and between crash and non-crash years. In adult females, selection on weight was stronger in crash years, a result which concurs with previous findings (Pelletier *et al*., 2007). In adult sheep of both sexes, selection on weight strengthened with increasing age, with heavy young sheep predicted to have only marginally higher annual fitness than lighter individuals, but heavier sheep enjoying far higher fitness as age increased. These models also revealed that senescence in annual fitness shows a more rapid decline in lighter adults than in heavy adults, which are able to maintain high annual fitness until death (Figure 5.5 and Figure 5.6a). It is known that annual fitness declines in the oldest females in this as in other populations (Wilson *et al*., 2009; Jones *et al*., 2008), due to senescence (both actuarial and reproductive). As ageing progresses and physiological systems deteriorate, the heaviest

females are still able to survive and/or reproduce, possibly as a result of greater resource availability, while lighter females cannot, resulting in strong positive selection on body weight. In young, healthy sheep, even lighter females are vigorous enough to survive and reproduce. Similarly, old males who are heavy can maintain investment in reproductive effort, while those who are lighter cannot.

5.5.4 Conclusions

Longitudinal, individual-based sampling in natural populations is difficult to accomplish, but it can reap rich rewards. For instance, by analysing selection on multiple traits over repeated episodes, a true picture of the target of selection can be gained (e.g. Milner *et al*., 1999a). Here, we have provided a rare investigation of selection acting on natural variation in a measure of parasite resistance in a wild mammal. Our findings suggest that there is significant negative selection on FEC only in lambs, once selection on the correlated trait of body weight is accounted for. We have also used our longitudinal data to show how selection patterns change across ages, and with environmental conditions. Such processes are of tremendous importance in age-structured populations inhabiting heterogeneous environments, but are rarely examined. This is in part because longitudinal sampling for parasites is difficult, and so it may be easier to take blood samples and analyse selection on measures of immune responsiveness (see Gustafsson *et al*., 1994; Raberg & Stjernman, 2003; Parejo & Silva, 2009, for rare examples). The first such analysis in the Soay sheep, of a heritable measure of antibody responsiveness, showed complex selection, with antagonistic selection via fecundity and survival (Graham *et al*., 2010). In this system it would be desirable to estimate selection on measures of immune responsiveness in August and selection via annual fitness, and the separate components of

fitness, which may be traded-off via immunity (e.g. Mills *et al*., 2010). More such studies would be welcome in aiding our understanding of natural selection on parasite resistance in natural populations, since only then will we begin to appreciate the extent of the influence of parasites on hosts in nature and their role in determining fitness and shaping life-history trade-offs between immunity, survival and reproduction.

Chapter 6

Phenotypic and genetic associations between natural parasite infections and host antibody responses in a wild mammal

6.1 Summary

Ecologists have long been interested in measuring investment in immunity in natural populations, particularly to estimate the cost of immune responses and trade-offs with other host life-history traits. However, global measures of immunity are often used which are not relevant to host-parasite interactions in the study population, or correlated with aspects of host condition or fitness, making interpretation of results difficult. In this study, we investigate the associations between an estimate of parasite infection intensity, an antibody with a wide array of specificities, and a parasite-specific antibody response in lambs and adult females in a natural population of Soay sheep. We found no evidence that parasite infection intensity was associated with either antibody response, but found that the antibody responses were positively correlated. There was considerable additive genetic variance in both antibody responses, which changed across ages in contrasting ways. Multivariate quantitative genetic analysis revealed that the correlation between antibody responses was largely due to residual effects in adults, but the genetic correlation was strong in lambs though marginally non-significant. The different traits also differed in their associations with body weight. The parasite-specific antibody response was positively associated with survival in lambs, but this was non-significant once the effect of a measure of parasite infection intensity was accounted for. The results reveal some of the links between antibody

responses, parasite resistance and physiological processes, and also provide the impetus for further analysis of selection on these traits through parasite resistance and tolerance.

6.2 Introduction

Individuals in natural populations are infected with a variety of parasites, with large variation between hosts in parasite resistance and tolerance to infection (Wilson *et al*., 2002; Raberg *et al*., 2009). Two common assumptions are that hosts harbouring more parasites have lower fitness, and that hosts with fewer parasites mount stronger immune responses and invest more resources in immunity (Graham *et al*., 2011). However, these assumptions may not apply in heterogeneous natural environments where hosts are nutrient-limited and vary widely in condition or health (Lazzaro & Little, 2009; Tompkins *et al*., 2011). The mammalian immune system and mechanisms of response to infection have been described in detail in laboratory studies (Murphy *et al*., 2008), and yet the immune responses of mammals in natural populations have been relatively poorly studied, with the exception of humans (e.g. Jackson *et al*., 2009b; McSorely & Loukas, 2010; Bourke *et al*., 2011). Studies in natural populations are important because they more closely reflect the situation in human populations where helminth infections are endemic than do laboratory populations of inbred mice with access to unlimited resources (Pedersen & Babayan, 2011). The majority of studies in natural populations have been on birds and have used global measures of immune function which may not be correlated with biologically relevant traits such as parasite infection intensity, individual condition, or where multiple measures have been used, even each other, and so meaningful interpretation of results may be difficult (Matson *et al*., 2006; Saks *et al*., 2006). It has been suggested that measures of immunity which are relevant to population-specific host-parasite interactions should be assayed, using the

immunological literature as a guide (Adamo, 2004; Bradley & Jackson, 2008). A recent paper has outlined a variety of approaches for employing data on parasite infection intensities, immune phenotypes, and host fitness in order to better understand associations between hosts and parasite in natural populations (Graham *et al*., 2011). In this study, we investigate the associations between an indicator of parasite infection intensity and two antibody responses in the free-living population of Soay sheep on the islands of St Kilda.

One of the key mechanisms contributing to observed heterogeneity in parasite infection intensities in natural populations is genetic heterogeneity in host susceptibility, and yet estimates of genetic variance in measures of parasite resistance or immune phenotype are extremely rare in natural populations (Wilson *et al*., 2002), with the exception of humans (Quinnell, 2003). The few published studies provide rare evidence that traits such as parasite resistance and immune responses have the potential to evolve (e.g. Coltman *et al*., 2001a; Beraldi *et al*., 2007; Pitala *et al*., 2007), but it is not known how these traits covary at the genetic, individual, or environmental level, which is crucial in understanding the evolutionary dynamics of phenotypic traits. Genetic correlations occur when linked genes influence multiple phenotypic traits, such that selection on one trait will lead to correlated selection on related traits (Lande, 1979). Estimates of correlations between traits associated with genetic and other sources of covariance can be gained from multivariate genetic analysis of phenotypic traits (Blows, 2007), which requires detailed data on phenotypic traits and relatedness. Such techniques have recently been used to study the genetic architecture of life-history traits in natural populations (e.g. Charmantier *et al*., 2006a). Such analysis could reveal whether there is a genetic basis to immune responses, whether there are synergistic or antagonistic genetic relationships between different types of immune response, and whether immune responses are genetically correlated with condition or parasite infection intensity. This would facilitate understanding of the processes governing associations between

host immune responses and parasites, as well as giving a more detailed picture of the potential for selection on such traits.

Infections with gastrointestinal helminth parasites are associated with reduced fitness in the St Kilda Soay sheep population (Gulland *et al*., 1993; Hayward *et al*., in press), but it is unknown how these parasites are associated with specific immune responses. Recently however, assays for several aspects of immunity have been conducted on stored blood samples, including anti-nuclear antibodies (hereafter ANA). ANA are a mixed population of autoantibodies that bind to an array of specific mammalian nuclear and cytoplasmic antigens and although they are implicated in autoimmune disease (Arbuckle *et al*., 2003) they are also associated with normal immune function (Digheiro & Rose, 1999). In the Soay sheep population, higher ANA levels are associated with increased probability of survival of harsh winters, but negatively associated with reproductive success in the previous breeding season (Graham *et al*., 2010). Here, we describe analysis of ANA and anti- *Teladorsagia circumcincta* antibody, which includes all antibody isotypes reactive to antigens of adult *T. circumcincta*, one of the most prevalent parasitic nematodes in the population. A key indicator of *T. circumcincta* infection intensity is the number of parasite eggs counted in faecal samples (faecal egg count, FEC, see below), and work in domesticated sheep has shown that this is reduced in resistant hosts by immunoglobulin A-mediated regulation of worm size and fecundity (Stear *et al*., 1995; Stear *et al*., 1997; Martinez-Valladares *et al*., 2005). However, it is unknown how specific antibody responses are associated with this estimate of parasite burden in this population, nor how measures of a parasite-specific immune response correlate with host condition or fitness, and this study is a first attempt to establish these relationships.

We tested for associations between FEC, ANA, and anti- *T. circumcincta* antibody (TcAb) using an eleven-year longitudinal data set, containing data on antibody responses, parasite infection, and life-history variables on over a thousand individuals, in lambs of both sexes and in adult females. Firstly, we tested for associations between the variables themselves using linear mixed-effects models, and accounted for associations with sex, age, and weight. We then performed univariate quantitative genetic analysis to partition the phenotypic variance in each trait into additive genetic and non-genetic sources of variation. We used a multivariate quantitative genetic analysis to test for covariance between traits, and to test for genetic correlations. Finally, we tested for associations between each of the three variables and survival. Immunity takes around six months to develop in domesticated sheep (Waller & Thomas, 1981; Smith *et al*., 1985), and so across age groups we expect to see this reflected in changes in both parasite burden and immune function. We predict that TcAb and ANA will show positive phenotypic and genetic correlations, since it is likely that antibody production will be associated with the same or linked genes (Covelli *et al*., 1989; Amor *et al*., 2005, and references therein). We also predict that the association between TcAb and FEC will be positive in lambs, since naïve lambs should produce antibody in proportion to the amount of antigen they encounter, and negative in adults, since TcAb should exert a protective effect and regulate FEC (Quinnell *et al*., 1995). We expect that ANA and FEC will be uncorrelated, since ANA include antibodies with a wide array of specificities which may be elicited by a variety of antigens. Finally, we predict that FEC will be negatively associated with survival, while the antibody measures will be positively associated with survival and indicative of good health.

6.3 Methods

6.3.1 Study population and data collection

The Soay sheep population of the St Kilda archipelago, NW Scotland (57°49′N 08°34′W), is thought originate from some of the first domesticated sheep in northern Europe, and to have been free-living on the island of Soay for several thousand years. The largest island of the archipelago, Hirta (638ha), was home to a human population until 1930, when they were evacuated due to their dwindling population size, and in 1932, 107 sheep were moved from Soay onto Hirta. The Hirta population has subsequently expanded, and the population inhabiting the Village Bay area of the island (around a third of the total sheep population and acreage) has been the subject of a longitudinal individual-based study since 1985 (Clutton-Brock & Pemberton, 2004). During the lambing season in April, around 95% of lambs are captured within a week of birth, given individual identification tags, and are blood sampled and weighed (Clutton-Brock & Pemberton, 2004). Maternities are assigned on the basis of behavioural interactions, and paternity is assigned using a pedigree constructed using the R package MasterBayes (Hadfield *et al*., 2006). Assignment of paternity is based on a combination of microsatellite and allozyme markers, and with fixed effects of horn type and age; paternity is assigned to a sire if that sire is predicted in at least 80% of the samples of the posterior distribution of the pedigree (Morrissey *et al*., submitted). Each August, around 60% of the Village Bay population are captured and weighed, measured, blood sampled, and faecal sampled. Blood samples are stored at 4°C, until centrifugation at 3000rpm for 15 minutes, within 24 hours of collection. Plasma is collected, aliquoted, and stored at -20°C until assayed. The majority of sheep captured produce a faecal

sample, which is stored at 4°C until it is examined for helminth parasite eggs. Blood and faecal samples assayed here were collected in Augusts during the period 1997 – 2007.

6.3.2 Parasitology and antibody assays

Faecal egg count

Faecal egg counts were conducted on August faecal samples using the McMaster egg counting technique. 3g of each faecal sample was placed in a tea strainer, and homogenized using a pestle whilst suspended in a bowl containing 87ml of saturated salt solution. The residual fluid was drained, and the contents of the bowl mixed with a pipette before filling both chambers of a McMaster slide, which has a marked grid, the volume of fluid under which is 0.30ml in total. Helminth eggs were then counted at 10x magnification, and the count was multiplied by 100 to give an egg count in eggs per gram of faeces. The most prevalent group of gastrointestinal parasites infecting the sheep are the directly-transmitted strongyle nematodes, a group of species largely consisting of *Teladorsagia circumcincta*, *Trichostrongylus axei*, and *Trichostrongylus vitrinus*. The eggs of these species and three others are indistinguishable by eye, and are counted together in the strongyle faecal egg count (hereafter referred to as FEC). Previous work on this population suggests that FEC is positively and linearly correlated with worm infection intensity (Gulland, 1992; Grenfell *et al.*, 1995; Wilson *et al.*, 2004). Heterogeneity in parasite genetics and stochastic variation in exposure are likely to contribute to variation in FEC. However, FEC also shows considerable variation due to differences between individuals; for instance, it shows substantial repeatability, with $r = 0.58 \pm 0.03$ across years in females, and $r = 0.42 \pm 0.07$ in males (Wilson *et al.*, 2004). There is also substantial variation in FEC due to genetics (Coltman

et al., 2001b; Beraldi *et al*., 2007), body size (Coltman *et al*., 2001a; Robinson *et al*., 2009), sex (Gulland & Fox, 1992; Wilson *et al*., 2004), and age (Craig *et al*., 2008; Hayward *et al*., 2009). The large amount of individual-level variation suggests that FEC may be treated as a trait of individual sheep, and indeed it is the indicator trait of choice in selective breeding for parasite resistance in domestic sheep populations (Sayers & Sweeney, 2005).

Anti-nuclear antibody:

A previous study assayed 2622 plasma samples taken from 1476 individual sheep in the Augusts of 1997-2007 (Graham *et al*., 2010). In a subset of 284 samples, it was shown that ANA is positively but quite weakly associated with three other antibody responses (RNP-specific antibody, anti- *T. circumcincta* antibody, and total immunoglobulin (Ig)G), indicating that ANA may reflect general levels of antibody responsiveness (Graham *et al*., 2010). The antibody assays were described in full by Graham *et al*. (2010, SOM); data from these assays are used in the present analysis:

Plasma samples were analysed with a REAADS ANA Test ELISA kit (Corgenix UK Ltd), modified for use in sheep with a polyclonal rabbit anti-sheep immunoglobulin secondary antibody (Ig/HRP P0163, Dako UK Ltd). Kit antigens consisted of various purified mammalian nuclear and cytoplasmic antigens derived from HEp-2 (Human Epithelial cell line 2) cells, including RNP, Sm, SSA, SSB, Scl-70, Jo-1, CENP-B, Ribosomal P, DNA, and histones. Thawed plasma samples were diluted 1:50 on ice, transferred in 100µl aliquots to antigen-coated ELISA plates, and incubated at 37°C for two hours. Wells were emptied, washed 4 times, and secondary antibodies were added at 0.1625µg/ml. Plates were incubated at 37°C for one hour,

and wells were emptied, washed four times, and 100µl of TMB/H_20_2 substrate were added per well. Plates were incubated at room temperature in the dark for 15 minutes, and 100µl of stop solution (1N sulphuric acid) were added per well. Absorbance (optical density, or O.D.) was then measured at 450nm on an Emax Precision Microplate reader (MDS Analytical Technologies, USA). Plasma-free blanks were run for both human- and ovine-specific secondary antibodies, and two controls were run on every 96-well plate: a Soay neonate "background control" sample with ovine-specific secondary antibody, and a human ANA-positive control with human-specific secondary antibody. Mean O.D. was 0.08 ± 0.01SD for plasma-free blanks, and 0.31 ± 0.08SD for neonate plasma. Controls were run in order to correct for variation in laboratory conditions on the rates of ELISA reactions, such as variation in ambient temperature. For each assay, a second sample was run on a different day, and the mean of the duplicates was taken for all statistical analysis.

To ensure that O.D. data were linearly related to the true concentrations of ANA, ANA titres were quantified by endpoint dilution analysis of a subset of the samples, consisting of the top 5% and bottom 1% of ANA O.D., since the highest values are most prone to saturation of the concentration curve (see Graham *et al*., 2010, SOM and references therein). A doubling dilution series from 1:50 to 1:400 was assayed for these samples, and titre was defined as the reciprocal of the last dilution at which sample O.D. was greater than background, plus 3SD. The O.D. reading in the original assays correlated strongly with the subsequent titre determination (rho = 0.72, p = <0.001), supporting the use of O.D. as a quantitative estimate of ANA concentration (Graham *et al*., 2010, SOM).

Anti-T. circumcincta *antibody:*

We measured antibodies of all isotypes against adult *T. circumcincta* (hereafter TcAb) in the same plasma samples as previously assayed for ANA. Plasma samples were analysed using a Sandwich ELISA: NUNC-immuno plates (Code: 439454, Maxisorp, Thermo Fisher Scientific) were coated with 50µl per well of adult *T. circumcincta* antigen (at a concentration of 2µg per ml of carbonate buffer), and incubated overnight at 4°C. Plates were emptied, before adding 200µl of 5% BSA:carbonate buffer and incubating for 2 hours at 37°C. Plates were washed five times with TBST using an automated plate washer. Sera were added to the first wells of each row of the plate, with one row on each plate used for a naïve blackface negative control (a sample from an individual animal of a domesticated breed, not exposed to parasites). A doubling dilution series was run across the plate, such that there were 12 dilutions of each sample per plate, from 1:50 to 1:1,024,000. Plates were then incubated for two hours at 37°C, and then washed 5 times with TBST using an automated plate washer. Detection antibody (Polyclonal Rabbit Anti-Sheep Immunoglobulins/HRP; DakoCytomation (Code no.: P0163)) was added (1:8000) at 100µl per well. Plates were then incubated for 1 hour at 37°C, washed 5 times with TBST, and twice with distilled water. TMB substrate was then added at 100µl per well, plates were allowed 3 minutes to develop, and the reaction stopped with 100µl of 1M HCl. Absorbance (optical density, or O.D.) was measured at 450nm on an Emax Precision Microplate Reader (MDS Analytical Technologies, USA). While this assay was intended to measure antibodies specific to adult *T. circumcincta*, there is likely to be considerable cross-reactivity with antigens of other parasites, including larval stages and other strongyle species (Williams *et al*., 2010).

6.3.3 Statistical analysis

We analysed 956 samples from lambs, with one sample per lamb, and 1018 samples from 406 adult females aged two and over (mean samples per female = 2.50, range = 1 – 11), collected in the Augusts of 1997-2007. We did not analyse yearlings or adult males due to the small number of samples from both of these subsets.

6.3.3.1 Response variables

Faecal egg count (FEC)*:* FEC provides an estimate of parasite infection intensity by counting the number of strongyle helminth eggs per gram of sheep faeces in a single sample. FEC plus one was natural log-transformed prior to analysis. Not all captured sheep produced a sample, and so any analysis incorporating FEC as a response or explanatory variable was conducted on a reduced data set of 831 samples from lambs, and 951 samples from 401 adult females.

Anti-nuclear antibody (ANA): The original O.D. reading from the ELISA assay was used as an estimate of the response strength. Each plasma sample was assayed twice, and the average O.D. of the two was then $\log_{10}$-transformed and multiplied by 100 to rescale for analysis. In all analyses of ANA, the plate-specific negative or "background control" (a plasma sample from a single Soay neonate) was included as a fixed effect.

Anti- ***T. circumcincta antibody*** **(TcAb):** Titre was defined as the reciprocal of the last dilution at which the sample O.D. was 3 S.D. greater than the assay date-specific mean negative control

(naïve blackface lamb) at dilution 1:200. This titre was then $\log_2$-transformed to conform to the assumptions of our statistical methods (see below).

6.3.3.2 Correlations between parasite resistance and antibody responses

We first tested for associations between FEC, ANA and TcAb using univariate linear mixed-effects model (LMMs) with each variable analysed in turn. In analysis of lambs, we fitted random effects of capture year to FEC, and capture year and assay date to ANA and TcAb; in analysis of adults, we fitted these random effects with the addition of individual identity in all models. Assay date was fitted as a random effect in order to account for variation due to laboratory conditions, such as temperature. Firstly, we analysed each of FEC, ANA, and TcAb, with both of the others as explanatory variables, plus effects of sex in lambs, age in adult females, August weight (WT hereafter) in both, and interactions. In lambs, we fitted model 1, and we fitted model 2 to adult females:

$$\begin{aligned} var_1 = \; & sex + WT + WT^2 + var_2 + var_2^2 + var_3 + var_3^2 + \\ & sex.WT + sex.var_2 + sex.var_3 + var_2.var_3 + sex.var_2.var_3 \\ & [\, + \text{random effects} \,] \end{aligned} \qquad \text{model 1}$$

$$\begin{aligned} var_1 = \; & age + age^2 + WT + WT^2 + var_2 + var_2^2 + var_3 + var_3^2 + TREATMENT + \\ & age.WT + age.var_2 + age.var_3 + var_2.var_3 + age.var_2.var_3 \\ & [\, + \text{random effects} \,] \end{aligned} \qquad \text{model 2}$$

where var_1, var_2, and var_3 refer to the three response variables. TREATMENT is a two-level factor, describing whether or not an adult female had been treated with anthelmintic drugs as part of experimental work at any point prior to FEC and antibody sampling (e.g. Gulland, 1992; Gulland *et al.*, 1993; Craig *et al.*, 2009). We did not test for any effect of treatment in lambs, since none are treated before their first August capture when faecal and blood samples are analysed.

We were also interested in potential trade-offs between growth and investment in immune function in lambs. We therefore ran a further set of models which included birth weight (measured in April), August weight (WT), and the interaction, which allowed us to test for an effect of growth as well as including fixed effects of both birth weight and body weight at the time of sampling. Lambs are caught up to a week after birth, during a period of rapid growth, so birth weight was calculated as the residuals from a regression of capture weight on capture age (BWT hereafter). Firstly, we fitted a simple model to each response variable with linear and quadratic effects of BWT and WT, and the interaction between the two (model 3; not shown). The interaction tests for an effect of growth while allowing testing for independent effects of BWT and WT. Next, we included effects of sex and other immunological variables, in an extension of the models described initially:

$$\begin{aligned} var_1 = \; & sex + BWT + BWT^2 + WT + WT^2 + var_2 + var_2{}^2 + var_3 + var_3{}^2 + \\ & sex.BWT + sex.WT + BWT.WT + sex.var_2 + sex.var_3 + var_2.var_3 + \\ & sex.BWT.WT + sex.var_2.var_3 \\ & [\, + \text{random effects} \,] \end{aligned} \qquad \text{model 4}$$

From the initial models described, the final model was reached by sequential removal of non-significant terms in order of least significance, with significance determined by F statistics on the appropriate degrees of freedom and with $\alpha = 0.05$.

6.3.3.3 Univariate quantitative genetic analysis

We next estimated the contribution of additive genetic effects to the phenotypic variance in each of the three traits by using a quantitative genetic 'animal model', which uses information from the population pedigree to estimate the contribution of relatedness to variance in trait values, a method developed in animal breeding (Henderson, 1950; 1975), and increasingly used in quantitative genetic analysis of natural populations (Kruuk, 2004). For each trait, we fitted the significant explanatory variables from the LMMs above as fixed effects, but excluded FEC or antibodies if they were significant, since we examined their relationships through the covariance structure of multivariate genetic analysis, described below. In analysis of lambs, we fitted variance components of additive genetic variance (V_A), year (V_Y), a maternal effect (V_M), and a residual effect (V_R), in models of all three variables, with the addition of assay date (V_{DATE}) in models of ANA and TcAb. For adult females, the variance components were the same, except that the individual effect was partitioned into the additive genetic component (V_A) and the permanent environment effect (V_{PE}), which estimates the effect of individual identity not explained by the pedigree; the sum of V_A and V_{PE} in adults is equal to the individual variance, or repeatability (V_{IND}). Heritability for each trait was estimated separately in lambs and adult females by dividing the additive genetic variance by the total phenotypic variance ($h^2 = V_A / V_P$). The significance of variance components was assessed from the change in log-likelihood (LogL) using likelihood ratio tests (LRTs), where the χ^2 test statistic is calculated as $-2*(LogL_{model2} -$

$LogL_{model1}$). The significance of the change in LogL due to dropping each variance component was assessed by calculating p-values based on the χ^2 statistic on one degree of freedom.

6.3.3.4 Trivariate quantitative genetic analysis

This set of models was designed to estimate the covariance between traits, and to determine whether any such covariance could be attributable to additive genetic effects or to other sources. Thus, FEC, ANA, and TcAb were fitted simultaneously as response variables in a multivariate 'animal model', and we included as fixed effects those terms which were significant in LMM analysis using model 1 and model 2, excluding other immune or parasitological variables. For lambs, we fitted V_A and V_R to all three variables, as well as V_{DATE} to ANA and TcAb. V_M was fitted to ANA only, since there was no maternal effect on FEC or TcAb, and V_Y was fitted to FEC and ANA only, since the annual variance in TcAb was negligible. In adult females, we fitted V_A, V_{PE}, and V_R to all three variables, as well as V_{DATE} to ANA and TcAb. We fitted V_Y to FEC and ANA only, since V_Y for TcAb was virtually zero. We did not include maternal effects because they were not significant in any of the univariate models of adult females. Where a variance component was fitted to all three response variables, it yielded a 3 x 3 variance-covariance matrix:

σ^2_{FEC}	$\rho_{FEC,ANA}$	$\rho_{FEC,TcAb}$
$\sigma_{FEC,ANA}$	σ^2_{ANA}	$\rho_{ANA,TcAb}$
$\sigma_{FEC,TcAb}$	$\sigma_{ANA,TcAb}$	σ^2_{TcAb}

where the diagonal elements are the variances of FEC, ANA and TcAb, the below-diagonal elements are the covariances between pairs of response variables, and the above-diagonal elements are the correlations between pairs rescaled from -1 to +1. We tested for significant covariance between FEC, ANA, and TcAb by constraining the covariance(s) of interest to zero and then comparing the fit of constrained models to the full model using LRTs (detailed below).

All of the above analysis was performed in ASReml 2.0 (VSN International, Hemel Hempstead, UK). For LMMs, we report parameter estimates for fixed effects ± 1SE; for univariate and trivariate animal models we report χ^2 statistics calculated from LRTs.

6.3.3.5 Associations with survival

A criticism of global measures of immune function typically used in ecological studies is that they are uncorrelated with parasite infection intensities and fitness and so the biological meaning of results may be difficult to interpret (Bradley & Jackson, 2008). We therefore tested for associations between FEC, ANA and TcAb measured in August, and lamb survival until May 15th the following year. We used generalized linear mixed-effects models (GLMMs) with binomial errors, and tested for linear and quadratic associations between each variable and survival separately. We then fitted all three variables as explanatory variables, with all two-way interactions. Finally, we analysed survival using a model with linear and quadratic terms for FEC, ANA, TcAb and weight, with sex as a two-level factor, and interactions between all variables, not including quadratic terms. Initial models were simplified by removing non-significant terms in the order of least significance, as determined by Wald F-statistics, and with α

= 0.05. Analysis was performed using the GLMM protocol in GenStat 13th Edition (VSN International, Hemel Hempstead, UK).

6.4 Results

We performed univariate and multivariate analysis of associations between FEC and two antibody responses (ANA and TcAb). Our results suggested that FEC was not associated with either of the antibody responses, but that antibody responses were positively correlated in both lambs and adult females. We found heritable variation in all three, and that covariance between antibody responses was due to residual effects in adults, and evidence for either or both residual and additive genetic covariance in lambs. Finally, the parasite-specific antibody was positively associated with survival in lambs, but did not predict survival as strongly as weight or FEC.

6.4.1 General observations

As expected, FEC was highest in male lambs, lower in female lambs, and much lower in adult females (Figure 6.1a). This was reversed with respect to ANA, which was much higher in adult females than in lambs of either sex (Figure 6.1b), and this was also the case for TcAb, which had a higher mean in adult females than in lambs of either sex (Figure 6.1c). This suggests that the lamb immune system is not fully functioning at four months of age, which is often cited as an explanation for the higher FEC generally observed in lambs both here and in domesticated sheep (Stear *et al*., 1996; Wilson *et al*., 2004).

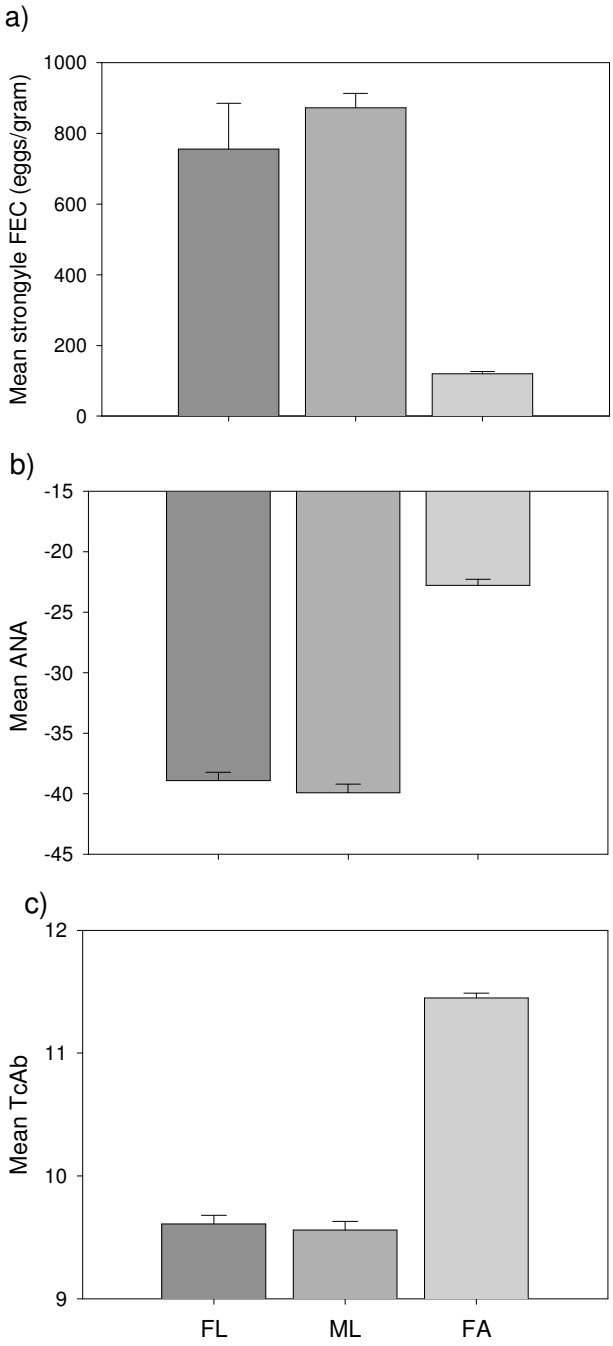

Figure 6.1: Adults and lambs show contrasting levels of parasite infection intensity and antibody responses: a) female lambs (FL) and male lambs (ML) have extremely high FEC in comparison to female adults (FA); b) ANA levels in lambs of both sexes are very low compared to those of female adults; c) levels of TcAb are much higher in female adults than in lambs of either sex. Bars show mean parasite counts and antibody levels in each subset +1SE.

6.4.2 Correlations between parasite resistance and antibody response

6.4.2.1 Lambs

The results of analysis of lambs using model 1 are shown in Table 6.1, and suggest that FEC is not correlated with either antibody measure, but that high levels of one antibody are associated with high levels of the other. FEC was significantly and negatively associated with WT, but was not significantly associated with either ANA (estimate = -0.0056 ± 0.0053, $F_{1,349} = 0.17$, p = 0.685) or TcAb (-0.0114 ± 0.0317, $F_{1,822} = 0.13$, p = 0.722). Despite the lack of a significant association with ANA, there was a significant interaction between ANA and sex which suggested that FEC increased with ANA in females, but decreased with ANA in males; however, it was not apparent whether either the increase or decrease differed significantly from zero (Table 6.1a). ANA was not significantly associated with FEC (0.3296 ± 0.2484, $F_{1,819} = 1.76$, p = 0.185), and did not differ significantly between the sexes (females = -0.2765 ± 0.6869, $F_{1,815} =$ 0.16, p = 0.689), but was significantly positively associated with TcAb (Table 6.1b). None of the interactions were significant. TcAb was not significantly associated with FEC (-0.0214 ± 0.0368, $F_{1,756} = 0.34$, p = 0.564), sex, or WT (Table 6.1c), but was significantly positively associated with ANA (Figure 6.2). There was also a significant interaction between sex and WT, which suggested that WT was positively associated with TcAb in females, but that TcAb showed no significant association with WT in males (Figure 6.3).

	Variable		Estimate	S.E.	F	d.f.	p
a)	***Faecal Egg Count***						
	Fixed effects						
	Intercept		9.7751	0.8460	134.08	1, 10	<0.001
	Sex						
		Female	-0.0407	0.2458	34.99	1, 821	<0.001
		Male	0.0000				
	Weight		-0.4717	0.1312	12.92	1, 823	<0.001
	Weight²		0.0141	0.0052	7.42	1, 823	0.007
	ANA		-0.0056	0.0053	0.17	1, 349	0.685
	Sex x ANA						
		Female	0.0138	0.0059	5.44	1, 819	0.021
		Male	0.0000				
	Variance components						
	Year		0.1695	0.0878			
	Residual		1.6973	0.0840			
b)	***Anti-nuclear antibody***						
	Fixed effects						
	Intercept		-69.3802	4.1847	274.90	1, 40	<0.001
	Negative		62.2374	9.1098	46.68	1, 31	<0.001
	TcAb		0.8763	0.2271	14.89	1, 938	<0.001
	Variance components						
	Year		31.2776	16.2904			
	Assay Date		2.9857	2.6422			
	Residual		99.9567	4.6233			
c)	***Anti-T. circumcincta antibody***						
	Fixed effects						
	Intercept		10.1746	0.3642	1015.87	1, 18	<0.001
	Sex						
		Female	-0.9680	0.4576	2.68	1, 938	0.103
		Male	0.0000				
	Weight		-0.0057	0.0227	2.61	1, 907	0.108
	ANA		0.0151	0.0038	15.38	1, 116	<0.001
	Sex x Weight						
		Female	0.0886	0.0354	6.24	1, 934	0.013
		Male	0.0000				
	Variance components						
	Year		0.0166	0.0461			
	Assay Date		0.2938	0.1100			
	Residual		1.8720	0.0870			

Table 6.1: Results of GLMM analysis of lambs using model 2a, where the response variable was a) FEC, b) ANA, and c) TcAb. Each table shows the final model for a particular trait, where only significant terms or main effects involved in significant interactions remain.

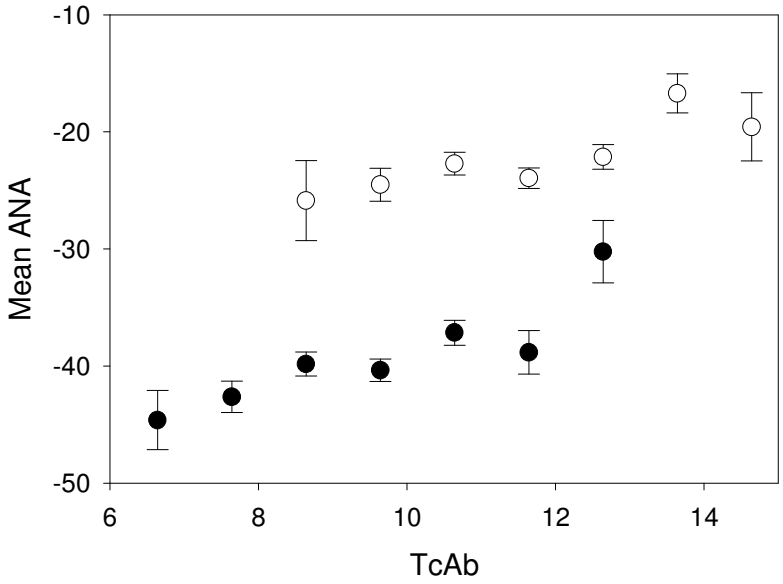

Figure 6.2: ANA was positively correlated with TcAb in both lambs (filled symbols) and adult females (open symbols). Points show mean TcAb for each bin of ANA, ± 1 S.E.

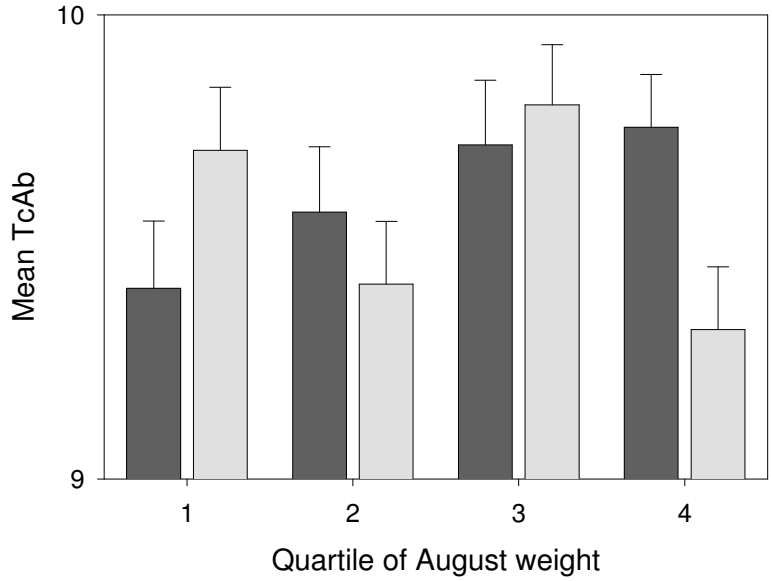

Figure 6.3: Anti- T. circumcincta antibody was positively associated with August body weight in female lambs (dark bars), but showed no strong association in male lambs (light bars). Bars show mean anti- *T. circumcincta* antibody in each quartile of August body weight, + 1 S.E.

6.4.2.2 Adult females

The results of model 2, testing for associations between FEC and antibody responses in adult females, are shown in Table 6.2. FEC followed a negative concave trajectory with age, as has been shown previously (Hayward *et al*., 2009), and was significantly and negatively associated with weight in a linear fashion. As was the case in lambs, FEC was not significantly associated with either ANA (-0.0059 ± 0.0067, $F_{1,277} = 0.79$, $p = 0.378$), or TcAb (0.0008 ± 0.0674, $F_{1,770} = 0.00$, $p = 0.999$), and none of the interactions were significant. ANA was positively associated with age, a relationship which has been shown to be due to greater longevity in individuals with high ANA levels, rather than to within-individual increases in ANA (Graham *et al*., 2010), and was significantly positively associated with TcAb. There was no significant association between ANA and FEC (-0.0778 ± 0.1122, $F_{1,738} = 0.48$, $p = 0.491$), and none of the interactions were significant. TcAb was significantly associated with FEC in a curvilinear manner which overall predicted high antibody production at higher FEC (Figure 6.4) and was linearly and positively associated with ANA (Figure 6.2). However, on removing the quadratic effect of FEC, the linear effect was non-significant (estimate = 0.0012 ± 0.0135, $F_{1,839} = 0.01$, $p = 0.932$). The significance of the quadratic effect appears to be based on high TcAb in some individuals with high FEC; however, sample size at this end of the range of FEC in adult females is small (only 55/951 FEC from females were ≥500) and so this result may be treated with caution.

	Variable	Estimate	S.E.	F	d.f.	p
a)	***Faecal Egg Count***					
	Fixed effects					
	Intercept	8.0427	0.8254	94.94	1, 472	<0.001
	Age	-0.7541	0.1410	28.60	1, 945	<0.001
	Age^2	0.0560	0.0110	26.09	1, 946	<0.001
	Weight	-0.1489	0.0379	15.39	1, 422	<0.001
	Variance components					
	Year	0.2149	0.1302			
	ID	0.7767	0.2474			
	Residual	5.6349	0.3209			
b)	***Anti-nuclear antibody***					
	Fixed effects					
	Intercept	-52.9213	4.2081	158.16	1, 116	<0.001
	Negative	52.1645	6.9061	57.06	1, 32	<0.001
	Age	0.6925	0.1404	24.32	1, 784	<0.001
	TcAb	0.6014	0.2595	5.37	1, 931	0.022
	Variance components					
	Year	20.6646	11.4169			
	Assay Date	4.6144	2.7304			
	ID	98.6818	8.9386			
	Residual	47.0136	2.7365			
c)	***Anti-T. circumcincta antibody***					
	Fixed effects					
	Intercept	11.5971	0.1354	7338.64	1, 57	<0.001
	FEC	-0.1496	0.0734	4.15	1, 840	0.043
	FEC^2	0.0277	0.0133	4.36	1, 851	0.038
	ANA	0.0064	0.0032	4.01	1, 238	0.047
	Variance components					
	Year	0.0150	0.0195			
	Assay Date	0.1506	0.0573			
	ID	0.7334	0.0843			
	Residual	0.7795	0.0472			

Table 6.2: Results of GLMM analysis of adult females using model 2b, where the response variable was a) FEC, b) ANA, and c) TcAb. Each table shows the final model for a particular trait, where only significant terms or main effects involved in significant interactions remain.

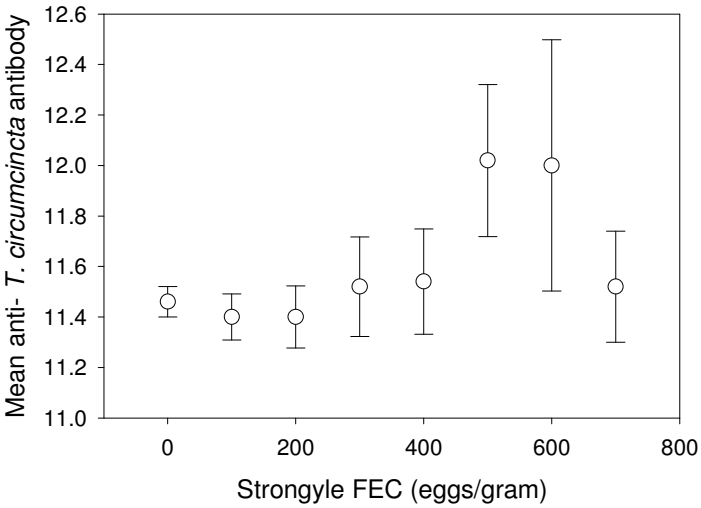

Figure 6.4 FEC was associated with TcAb in adult females in a non-linear fashion, but the linear effect of FEC was non-significant. Points show mean TcAb at each FEC interval ± 1S.E., where zero is zero, 100 is greater than zero and less than or equal to 100, continuing until 700, which is representative of all FEC samples greater than 600. The large standard errors associated with higher FECs are indicative of small sample size, and so the significance of the association between FEC and TcAb should be treated with caution.

6.4.2.3 Associations with weight and birth weight

The results of analysis of FEC using model 3 suggested that BWT was not associated with FEC (estimate = -0.0951 ± 0.0701, $F_{1,604} = 1.84$, p = 0.176), but that lambs that were heavier in August had lower FEC (WT = -0.6021 ± 0.1321, $F_{1,824} = 20.78$, p = <0.001; $WT^2 = 0.0204 \pm 0.0052$, $F_{1,825} = 15.62$, p = <0.001). BWT was significantly negatively associated with FEC if WT was excluded from the model (-0.2043 ± 0.0525, $F_{1,606} = 15.16$, p = <0.001); this result can be explained by the positive correlation between BWT and WT, and this result also suggests that WT is a better predictor of August FEC than is BWT. The results of model 4 showed that neither BWT nor the interaction were significantly associated with FEC, and the final model had the same structure as that in Table 6.1a.

ANA was not significantly associated with BWT (0.0910 ± 0.5220, $F_{1,689} = 0.03$, p = 0.862), WT (-0.1233 ± 0.1284, $F_{1,945} = 0.01$, p = 0.924), or the interaction (-0.0524 ± 0.2888, $F_{1,682} = 0.03$, p = 0.857), when analysed using model 3, and model 4 reduced to the same as that shown in Table 6.1b, with only TcAb significantly associated with ANA.

TcAb was not significantly associated with the interaction between BWT and WT in analysis using model 3 (0.0369 ± 0.0271, $F_{1,678} = 1.86$, p = 0.173). However, it was significantly associated with August weight (WT = 0.3479 ± 0.1553, $F_{1,685} = 5.02$, p = 0.026; WT^2 = -0.0121 ± 0.0059, $F_{1,685} = 4.27$, p = 0.040), which suggested a convex curve with low TcAb at low and high weights, but removing the quadratic effect showed that the linear effect of WT was non-significant (0.0320 ± 0.0271, $F_{1,669} = 1.39$, p = 0.239). TcAb was independently associated with BWT (-0.1982 ± 0.0724, $F_{1,673} = 7.49$, p = 0.007), which suggested that light-born lambs had higher TcAb in August. However, analysis using model 4 suggested that TcAb was not significantly associated with WT (0.0314 ± 0.0312, $F_{1,593} = 1.02$, p = 0.314), and was only significantly associated with BWT (-0.1194 ± 0.0539, $F_{1,683} = 4.9$, p = 0.028) and ANA (0.0146 ± 0.0046, $F_{1,117} = 10.11$, p = 0.002).

6.4.3 Univariate animal models

The results of genetic variance components analysis of lambs is shown in Table 6.3a, and the proportion of phenotypic variance accounted for by each variance component is shown in Figure 6.5a. There was additive genetic variance in all three variables, which was not significant for FEC, accounting for only 4.15% of phenotypic variance, but accounted for 10.73% and 31.17% of phenotypic variance in ANA and TcAb respectively, and was significant in both. There was

significant year-to-year variation in FEC and ANA, where it accounted for 8.96% and 24.19% of phenotypic variance respectively, but not in TcAb, where it accounted for only 0.70%. Date of assay was significant in models of ANA and TcAb, and accounted for 2.24% and 15.13% of phenotypic variance respectively. The maternal effect was zero for FEC, and the maternal effect for TcAb was marginally non-significant, accounting for 5.90% of the phenotypic variance. There was a significant maternal effect on ANA, which accounted for 8.51% of the total phenotypic variance.

Table 6.3b shows the results of genetic variance components analysis of adult females, and Figure 6.5b shows the proportion of phenotypic variance accounted for by each variance component. Additive genetic variance accounted for a significant portion of the total phenotypic variance of ANA (27.06%) and TcAb (17.30%), but it only accounted for a smaller and non-significant proportion of the variance in FEC (6.30%). Including the permanent environment effects and additive genetic effects, the repeatabilities of the three traits were 0.11 ± 0.03 for FEC, 0.51 ± 0.06 for ANA, and 0.40 ± 0.06 for TcAb. There was significant annual variation in FEC and ANA, accounting for 3.27% and 12.08% of total phenotypic variance respectively, but annual variance only accounted for 0.10% of phenotypic variance in TcAb. There was significant variance associated with assay date for ANA and TcAb, comprising 2.52% and 8.28% of phenotypic variance respectively. The maternal effect was zero for FEC, and accounted for only 2.66% and 0.58% of phenotypic variance in ANA and TcAb respectively, and remained non-significant.

	Variable	Mean	S.D.	V_Y	V_A	V_{PE}	V_M	V_{DATE}	V_R	h^2 (± SE)
a)	***Lambs***									
	FEC	6.0597	1.4470	0.1681***	0.0778	-	0	-	1.6295	0.04 (0.06)
	ANA	-39.4188	15.4172	0.4453***	14.8444*	-	11.7759**	3.1019*	75.1783	0.11 (0.06)
	TcAb	9.5884	1.4751	0.0157	0.6968***	-	0.1318	0.3379***	1.0514	0.31 (0.08)
b)	***Adult females***									
	FEC	2.6619	2.6434	0.2165***	0.4175	0.354	-	-	5.6423	0.06 (0.05)
	ANA	-22.7924	15.6422	20.2607***	45.3980***	47.0397	4.4607	4.2281***	46.3849	0.27 (0.09)
	TcAb	11.4503	1.2955	0.0016	0.2909*	0.4467	0.0097	0.1393***	0.7934	0.17 (0.08)

Table 6.3: Results of variance components analysis for a) lambs and b) adult females. Presented are the variance components estimates from the initial model where all variance components with a numerical value were included. Abbreviations for each variance component are described in the text. Significance of each variance component was evaluated by dropping the term from the full model and comparing the reduced model to the full model and assessing the change in log-likelihood using LRT; significance is denoted as *** $p < 0.001$, ** $0.001 \leq p < 0.01$, * $0.01 \leq p \leq 0.05$.

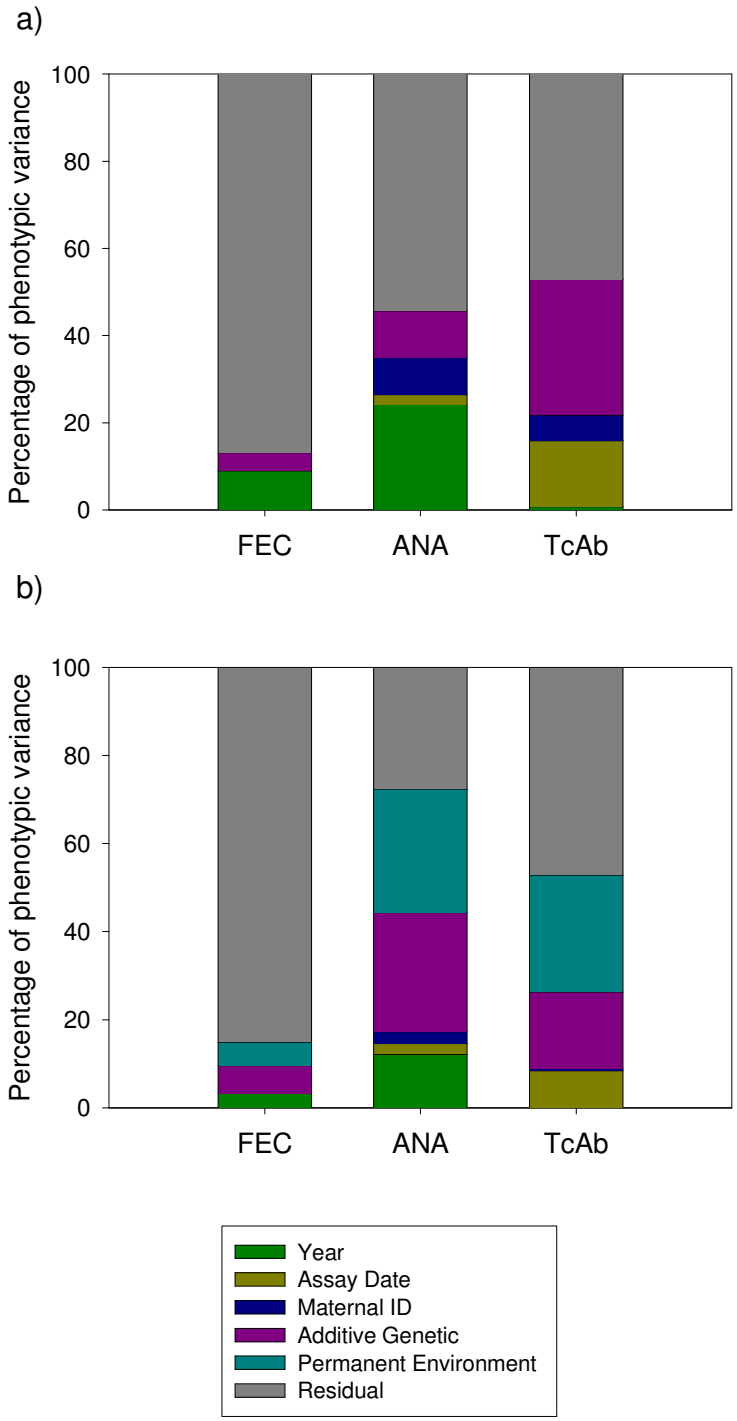

Figure 6.5: a) the proportion of phenotypic variance in FEC, ANA, and TcAb of lambs explained by each variance component, after correcting for fixed effects significant in Table 1a, b, and c. For the magnitude and significance of different components, see table 3a; b) the proportion of phenotypic variance in FEC, ANA, and TcAb of adult females explained by each variance component, after correcting for fixed effects significant in Table 2a, b, and c. For the magnitude and significance of different components, see table 3b.

6.4.4 Trivariate animal models

Firstly, we ran trivariate models without additive genetic effects, and found significant but relatively weak positive residual covariance between ANA and TcAb in both lambs (χ^2_1 = 13.98, p = <0.001, r_P = +0.1383) and adult females (χ^2_1 = 4.94, p = 0.026, r_P = +0.0916). The residual covariance between FEC and ANA was also positive and marginally significant, although it was weak (χ^2_1 = 3.94, p = 0.047, r_P = +0.0789). None of the other residual covariances were significant in lambs or adults, and none of the individual covariances were significant in adult females.

We attempted to partition the covariances between variables in lambs into their genetic and residual components by adding the population pedigree and additive genetic effects. The results of genetic trivariate analysis of lambs are shown in Table 6.4; models referred to in this section can be found in this table. Firstly, we constrained all of the residual covariances (trivariate model 2 in Table 6.4), and found a small and non-significant change in model fit; we then constrained all of the genetic covariances (model 3), and although the change was non-significant, the χ^2 statistic indicated that if most of this change was due to one of the covariances, it could reach significance. In order to determine which, if any, of the pairs of variables showed significant covariance, we next constrained both the residual and genetic covariances of each pair of variables in turn. There was no evidence for significant covariance between FEC and ANA (trivariate model 4) or FEC and TcAb (model 5), but there was significant covariance between ANA and TcAb (model 6). To determine whether this was due to residual or genetic effects, we constrained each covariance component to zero in turn (models 7 – 12). None of these were significant, but model 12 suggested there was a positive and marginally non-significant genetic covariance between ANA and TcAb (χ_1^2 = 3.56, p = 0.059, r_G = +0.4343). This suggests that there is significant covariance

between antibody measures, but that the model is unable to separate residual and genetic effects, although the genetic correlation is much stronger and approaches significance.

Model	Model description	LogL	χ^2	d.f.	p
1	Full Unconstrained	-3792.97			
2	Constrain all residual σ(x,y)	-3794.44	2.94	3	0.401
3	Constrain all genetic σ(x,y)	-3795.55	5.16	3	0.160
4	Constrain residual and genetic σ(FEC,ANA)	-3795.01	4.08	2	0.130
5	Constrain residual and genetic σ(FEC,TcAb)	-3793.62	1.30	2	0.522
6	Constrain residual and genetic σ(ANA,TcAb)	-3800.40	14.86	2	<0.001
7	Constrain residual σ(FEC,ANA)	-3794.10	2.26	1	0.133
8	Constrain residual σ(FEC,TcAb)	-3793.37	0.80	1	0.371
9	Constrain residual σ(ANA,TcAb)	-3793.08	0.22	1	0.639
10	Constrain genetic σ(FEC,ANA)	-3793.00	0.06	1	0.806
11	Constrain genetic σ(FEC,TcAb)	-3793.62	1.30	1	0.254
12	Constrain genetic σ(ANA,TcAb)	-3794.75	3.56	1	0.059

Table 6.4: Results of trivariate quantitative genetic analysis of FEC and antibody responses in lambs, where Model 1 is the full unconstrained model described in the text. The significance of covariance components were tested by comparing the full model to models with covariance components of interest constrained to zero as indicated in the second column. The χ^2 test statistic was calculated using LRTs as described in the text, and significance was determined from the χ^2 distribution on the degrees of freedom indicated, which is equal to the number of covariance components constrained in comparison to the full model.

The results of trivariate models for adult females are shown in Table 6.5, where models referred to in this section are described. We first compared the full unconstrained model to models with all residual covariances constrained (trivariate model 2 in Table 6.5), and there was no significant change in model fit. We then constrained all of the permanent environment and additive genetic covariances (hereafter referred to as the individual covariance), and found no significant change in model fit (model 3). We then constrained all of the additive genetic covariances (model 4), which only induced a small and non-significant change in model fit. We next constrained all covariances between each pair of response variables in turn; there was no evidence for significant covariance between FEC and ANA (model 5) or FEC and TcAb (model 6), but there was a marginally non-significant

drop in model fit when all covariances between ANA and TcAb were constrained (model 7). Finally, we constrained each of the residual, individual, and genetic covariances in turn (models 8 – 16). All were non-significant, with the exception of a significant but weak positive residual covariance between ANA and TcAb ($\chi_1^2 = 4.88$, $p = 0.027$, $r_P = +0.0909$).

Model	Model Description	LogLik	χ^2	d.f.	p
1	Full unconstrained	-4821.92			
2	Constrain all residual σ(x,y)	-4824.73	5.62	3	0.132
3	Constrain all individual σ(x,y)	-4824.66	5.48	6	0.484
4	Constrain all genetic σ(x,y)	-4823.23	2.62	3	0.454
5	Constrain all σ(FEC,ANA)	-4822.73	1.62	3	0.655
6	Constrain all σ(FEC,TcAb)	-4823.38	2.92	3	0.404
7	Constrain all σ(ANA,TcAb)	-4825.41	6.98	3	0.073
8	Constrain residual σ(FEC,ANA)	-4822.00	0.16	1	0.689
9	Constrain residual σ(FEC,TcAb)	-4822.23	0.62	1	0.431
10	Constrain residual σ(ANA,TcAb)	-4824.36	4.88	1	0.027
11	Constrain individual σ(FEC,ANA)	-4822.46	1.08	2	0.583
12	Constrain individual σ(FEC,TcAb)	-4823.32	2.80	2	0.247
13	Constrain individual σ(ANA,TcAb)	-4822.58	1.32	2	0.517
14	Constrain genetic σ(FEC,ANA)	-4821.99	0.14	1	0.708
15	Constrain genetic σ(FEC,TcAb)	-4822.52	1.20	1	0.273
16	Constrain genetic σ(ANA,TcAb)	-4822.49	1.14	1	0.286

Table 6.5: Results of trivariate quantitative genetic analysis of FEC and antibody responses in adult females, where Model 1 is the full unconstrained model described in the text. The significance of covariance components were tested by comparing the full model to models with covariance components of interest constrained to zero as indicated in the second column. The χ^2 test statistic was calculated using LRTs as described in the text, and significance was determined from the χ^2 distribution on the degrees of freedom indicated, which is equal to the number of covariance components constrained in comparison to the full model.

6.4.5 Associations with survival

When considering associations between individual variables and survival, lambs with higher FEC experienced lower survival (FEC = 0.6144 ± 0.0.2074, $F_{1,804} = 8.78$, $p = 0.003$; $FEC^2 = -0.0960 \pm 0.0242$, $F_{1,805} = 15.71$, $p = <0.001$). ANA was not associated with survival (ANA =

0.0084 ± 0.0088, $F_{1,910} = 0.91$, p = 0.339), but lambs with higher TcAb had higher survival over the subsequent winter (TcAb = 0.1267 ± 0.0606, $F_{1,927} = 4.38$, p = 0.037). However, once all three variables were included in the same model, the effect of TcAb was non-significant (TcAb = 0.0999 ± 0.0637, $F_{1,804} = 2.46$, p = 0.117), and only FEC was a significant predictor of survival, as before. We then added effects of sex and weight to the model and found that males were predicted to have lower survival (males = -1.0739 ± 0.2229, $F_{1,801} = 23.22$, p = <0.001), and that heavier lambs survived better (weight = 0.2620 ± 0.0473, $F_{1,804} = 30.70$, p = <0.001). As above, FEC was significantly associated with survival (FEC = 0.5312 ± 0.2240, $F_{1,802} = 5.62$, p = 0.018; FEC^2 = -0.0705 ± 0.0262, $F_{1,803} = 7.22$, p = 0.007), but neither of the antibody responses were significantly associated. The last term to be removed from the model was a marginally non-significant interaction between FEC and sex, which indicated that the negative association between FEC and survival was marginally stronger in male lambs than in females (FEC x male = -0.3970 ± 0.1738, $F_{1,798} = 3.82$, p = 0.051).

6.5 Discussion

We tested for associations between an estimate of parasite infection intensity (FEC) and two antibody responses (ANA and TcAb) in a free-living mammal population, and also for effects of these on survival of lambs. We found no strong associations between FEC and either the general or parasite-specific antibody responses, but found that the two antibody responses were positively associated in both lambs and adult females. There was significant additive genetic variance for both antibody measures, but we were unable to find conclusive evidence of genetic covariance between these, although the genetic covariance approached significance in lambs.

6.5.1 Phenotypic associations between parasite resistance and antibody responses

In the introductory section, we predicted no association between FEC and ANA, but that FEC would be correlated with TcAb. FEC is not associated with ANA in adult females (Graham *et al*., 2010), and we confirmed this here while also finding that there was no association between the two in lambs. The ubiquity of ANA, which is made up of an array of antibodies with a range of specificities (Dighiero & Rose, 1999), perhaps makes this result unsurprising. However, we did not find the predicted association between FEC and TcAb, which we expected either to be positively associated (indicative of an antibody response made in proportion to exposure), or negatively correlated (indicative of a response with a regulatory effect on worm fecundity). A possible explanation for this is that the relationship between infection intensity and FEC may be non-linear, and so, for instance, hosts with low FEC could either harbour very few worms or very many, which may be associated with a very high or very low antibody response. Evidence for a linear relationship between FEC and worm number in this population comes from post-mortem counts of sheep dying over winter (Gulland *et al*., 1992; Grenfell *et al*., 1995). Mortality is due to protein malnourishment (Gulland, 1992), and these sheep are likely to be in very poor condition and unable to mount an effective immune response. It may be that in healthy sheep the density-dependent relationship between worm size and worm number seen in mainland sheep and many other helminth parasites is the norm (Stear & Bishop, 1999; Bishop & Stear, 2000), which may make the association between antibody responses and an indicator trait such as FEC very complex.

Another explanation may be that TcAb is too specific to correlate with FEC, since it is the response to only a single worm species out of six from five different genera (Wilson *et al*.,

2004). Despite the potential cross-reactivity of antibodies to these species, the fact that the composition of strongyle species varies across age and sex groups and between individuals means that *T. circumcincta* does not comprise the bulk of adult worms in every infection (Craig *et al*., 2006). This means that in any given individual, FEC will be regulated to a certain extent by TcAb, but may be regulated even more by antibodies specific to other worm species. This is likely to be particularly pertinent in lambs, where on average only 18% of adult female strongyles are *T. circumcincta*; this increases to 22% in yearlings, 55% in 2-year-olds and 49% in adults (Craig *et al*., 2006).

As we predicted, in both lambs and adult females, there was a positive association between the general antibody response (ANA) and the parasite-specific antibody response (TcAb). This could suggest that genetically resistant hosts mount strong antibody responses of all kinds (Stear *et al*. 1999; Quinnell, 2003; Stear *et al*., 2009), or that individuals exposed to one antigen are more likely to be exposed to other types, something which we attempted to establish using the trivariate analyses which are discussed below. Another explanation, linked to the first, is that both covary positively with condition, health, or resource acquisition, since immune function is likely to be a condition-dependent trait, particularly in heterogeneous, resource-limited environments (Lazzaro & Little, 2009).

6.5.2 Associations with body weight

In the Soay sheep, body weight is positively associated with a range of fitness traits, including survival, reproductive success, and parasite resistance (Coltman *et al*., 2001a; Craig *et al*., 2009; Wilson *et al*., 2009). Heavier sheep are therefore considered to be in better health and more vigorous, although body weight is not a measure of condition *per se*. As expected, FEC in lambs was negatively associated with BWT (Figure 6.7a), although this

was not significant when the negative association with August weight (Figure 6.6a) was accounted for; FEC was also negatively associated with WT in adult females. ANA was uncorrelated with both BWT and WT in lambs, which was unsurprising given that ANA was uncorrelated with WT in adult females both here and in the analysis of Graham *et al*. (2010). TcAb, although uncorrelated with WT in adult females, was significantly and independently associated with WT and BWT in lambs. The non-linear association with WT suggested that the heaviest and lightest lambs had low TcAb (Figure 6.6b), while BWT was negatively associated with TcAb, suggesting that heavy-born lambs had low TcAb during summer (Figure 6.7b). The lambs with lowest WT may be the very latest-born and youngest individuals, and so may be underdeveloped compared to older lambs which may mount developing antibody responses (Waller & Thomas, 1981; Smith *et al*., 1985); the same pattern will not be apparent with BWT, since late-born individuals will not necessarily be lighter. The group of the heaviest individuals mounting low responses are all males, which are generally observed to experience higher parasitism and invest less in immunity than females (Klein, 2004), and this was reflected by the observation that WT was non-significant in the results of model 4 for lambs, where sex was included in the model.

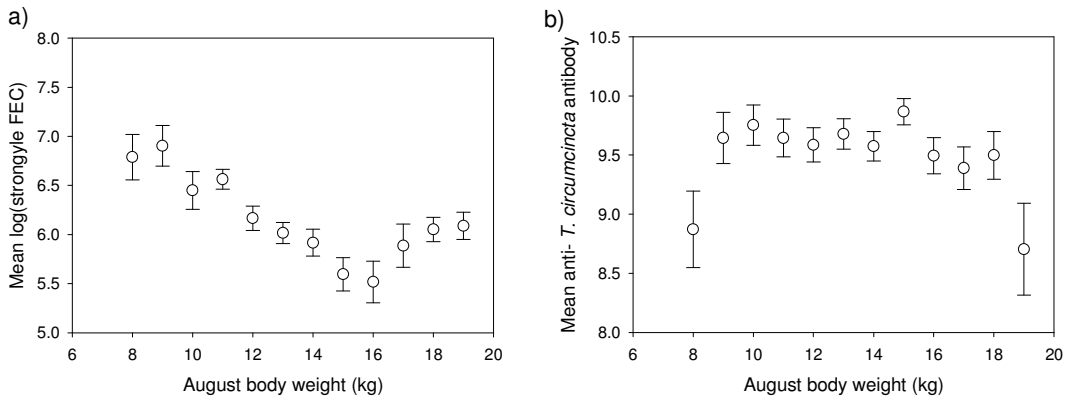

Figure 6.6: August body weight was significantly associated with both a) FEC, and b) anti- *T. circumcincta* antibody in lambs. Note that although the quadratic association with TcAb is significant, this appears to be due to low responses in the heaviest and lightest sheep, since the linear association is non-significant (see Results). Points show mean FEC and TcAb at each weight interval ± 1S.E., where 8 is less than or equal to 8, 9 is greater than 8 and less than or equal to 9; this continues until 19, which includes data from lambs heavier than 18kg.

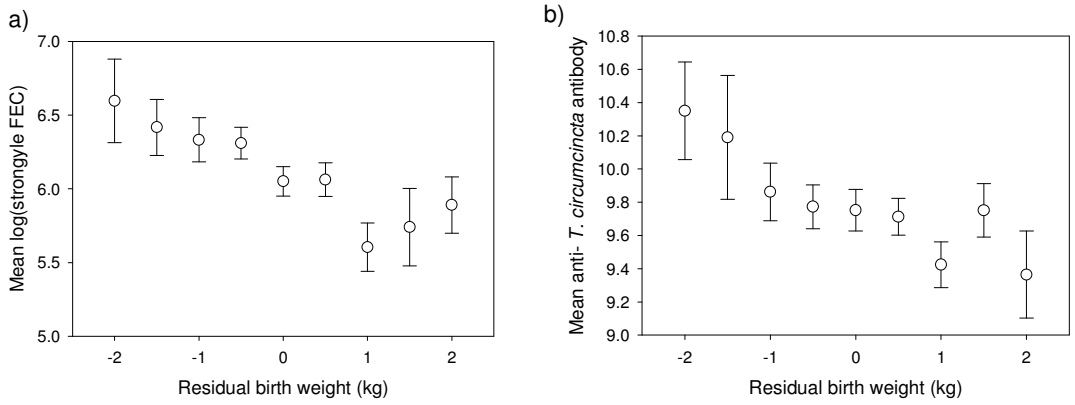

Figure 6.7: Residual birth weight (BWT) in lambs was positively associated with both a) FEC and b) TcAb. The association with FEC was non-significant in the presence of August body weight, suggesting that this predicts FEC better than BWT. However, BWT appears to predict TcAb better than WT. Points show mean FEC and TcAb at each BWT interval, where -2 is less than or equal to -2, -1.5 is greater than -2 and less than or equal to -1.5; this continues until 2, which includes data from lambs with BWT greater than 1.5.

However, it is certainly true that there is no strong linear association between August weight and TcAb in lambs, while there is a strong relationship between BWT and TcAb. This cannot be explained by sex differences since males are on average only 4% heavier than females at birth (Clutton-Brock *et al.*, 2004a), and there was no effect of sex in any analysis of TcAb. A possibility is that TcAb and FEC could both be correlated with a hidden variable such as number of adult *T. circumcincta* but uncorrelated with each other. The importance of BWT in determining August TcAb suggests that maternal effects are likely to be important, and work investigating such effects is currently ongoing.

These results suggest that FEC is more mass-dependent than either of the antibody responses. This could be because harbouring worms is directly associated with weight loss (Craig *et al.*, 2008), perhaps through reducing nutrient assimilation (Gulland, 1992), and because of the lack of association between antibody responses and FEC, antibody responses are not associated with weight. The relationship between FEC and weight could be a way of

investigating tolerance in future analysis, something which has been rarely investigated in natural populations, but could be of crucial importance (Raberg *et al*., 2007; 2009; Graham *et al*., 2011).

6.5.3 Contributions of additive genetic variance

Quantitative genetic analysis revealed additive genetic variance for all three traits, but this was not significant for FEC in either lambs or adults. Previous analysis in this population has suggested that heritability estimates depend on the data and analytical methods used and the model formula (Smith *et al*., 1999; Coltman *et al*., 2001a; Beraldi *et al*., 2007). We found a repeatability of FEC in adult females of only 0.11 ± 0.03 in the present study, compared with 0.58 ± 0.03 previously (Wilson et al., 2004; however, we accounted for body weight and age in our estimates, which are both likely to explain a large amount of the variation. In a previous study, the heritability of ANA was estimated at 0.12 ± 0.06 in lambs and 0.26 ± 0.09 in adult females (Graham *et al*., 2010 SOM), which is in agreement with the results presented here (Table 6.3). A new finding in the current work was significant additive genetic variance for anti- *T.circumcincta* antibody, with heritability estimates of 0.31 ± 0.09 and 0.14 ± 0.07 in lambs and adult females respectively. This extremely substantial estimate is in line with previous estimates of IgA activity from naturally-infected domesticated Scottish Blackface lambs, which ranged from 0.38 to 0.56 at various times of year (Strain *et al*., 2002). This result suggests that there is the potential for selection on specific antibody responses in this population, such that lambs which develop their own immunity quickly during their first summer could reduce their parasite burdens by the winter and experience greater survival prospects, as suggested by the results in chapter 5 (Hayward *et al*., in press).

It is interesting to note the changes in genetic variance with age in the two antibody responses, and how this relates to the evidence for selection thus far. ANA had a lower heritability in lambs, and there was no evidence to suggest that it was under selection via survival, while the heritability was higher in adults, in which it is positively associated with survival (Graham *et al*., 2010). Meanwhile, TcAb had a higher heritability in lambs than in adults, and was associated with survival, even though this effect was masked once FEC was accounted for, while TcAb was not associated with survival in analysis of a subset of samples in a previous analysis (Graham *et al*., 2010). The manner in which the genetic variance in ANA could be maintained, through negative associations with fecundity and positive associations with survival, has been discussed by Graham *et al*., 2010). A more thorough analysis of selection on TcAb in lambs may be required to determine the mechanisms behind the high genetic variance in this trait. Trade-offs with other immune responses, such as those involved in microparasite resistance could be implicated, but more analysis will be needed.

6.5.4 Covariance between traits

A preliminary trivariate model with no genetic term suggested a weak but significant residual correlation between ANA and TcAb of around +0.1 in both lambs and adults. This was apparently due to residual effects in adult females, but unfortunately, we were unable to discern whether this was due to additive genetic covariance or residual covariance in lambs. The genetic covariance was closer to significance and the correlation stronger than the residual, suggesting that a genetic effect is likely to be important, but we were unable to confirm this statistically. Genetic resistance is a trait commonly discussed in veterinary parasitology, with the aim of breeding sheep for elevated resistance or tolerance (Strain *et al*., 2002; Martinez-Valladares *et al*., 2005; Stear *et al*., 2009), and it is also known that host

genetics may have a large impact on resistance to parasites in natural populations (Duncan & Little, 2007; Lazzaro & Little, 2009), although estimates of genetic variance in resistance are rare. Thus it may be that genes for antibody production are linked or closely associated within the genome, and that individuals that tend to produce high levels of one antibody tend to produce high levels of other types (Covelli *et al*., 1989; Edfors-Lilja *et al*., 1998; Amor *et al*., 2005). The covariance between antibody measures in adult females did not have a substantial genetic component, and only the residual covariance was significant. This could be explained by factors such as exposure; for instance, hosts exposed to large amounts of worm antigen could produce a large amount of both antibodies, with hosts exposed to few worms producing a smaller amount of both antibodies. A further explanation is that the correlation could be due to some other host characteristic such as weight or condition, which varies with environmental conditions. Hosts in better condition could mount stronger antibody responses, possibly as a result of greater resource availability; this condition-dependence could be another explanation for their high genetic variance (Rowe & Houle, 1996).

6.5.5 Antibody responses and survival

Our predictions regarding associations between FEC, antibody responses, and survival were partially supported. Previous work has shown that August ANA is positively associated with survival in adult females, but that there is no survival benefit of TcAb (Graham *et al*., 2010, SOM). In lambs, we found evidence to suggest the opposite. TcAb was positively associated with survival, but not when either FEC or WT were added to the model as predictors, suggesting that these are more closely associated with survival. While TcAb is indicative of the response to one worm species, FEC is an indicator of infection with the whole range of strongyle species, and while mounting a strong antibody response could have a survival

effect, individual condition and parasite burden are more broad-scale indicators of condition and survival prospects. This raises the question of the biological significance of TcAb. It is certainly involved in a biologically relevant host-parasite interaction, which is the basis for its choice as an indicator of immune phenotype (Adamo, 2004; Bradley & Jackson, 2008; Pedersen & Babayan, 2011), but is apparently not strongly related to body weight, survival, or parasite infection intensity. Perhaps it is too specific, since in this population, as in all natural populations, hosts stand a high chance of being infected with more than one parasite species, which may be met with different or even conflicting immune phenotypes which may constrain the effectiveness of any response (Ardia *et al*., 2011). A possible solution could be the use of a multivariate approach, assaying for a variety of functionally related immune phenotypes and using this to gain a single measure of functionally relevant responses (Bradley & Jackson, 2008); for instance, assaying cytokines characteristic of different arms of the immune response (Graham *et al*., 2007; Pedersen & Babayan, 2011). This could provide a measure of the response made to the prevalent helminth parasites, without focusing on a single one which is likely to be present to varying degrees across individual hosts.

6.5.6 Conclusions

In this study, we have described associations between measures of parasite burden and antibody responses in a free-living population of Soay sheep. Our results illustrate the complexity of the immune system and studying it in an ecological context, since neither general nor specific antibody responses were correlated with an estimate of parasite infection intensity. We have also shown that specific antibody responses show a significant proportion of additive genetic variance, and that antibody measures covary at the phenotypic level, such that some individuals mount strong antibody responses, while others mount weaker responses. We found some evidence for a genetic correlation between functional antibody

responses in lambs, but not in adult females; however, even the correlation in lambs was marginally non-significant, emphasizing the need for large detailed datasets when performing multivariate genetic analysis. We have therefore seemingly failed to choose relevant immune response variables, despite choosing a general measure and a parasite-specific response. How, then, should the strength or efficacy of the immune response to helminths be assessed in natural populations? A longitudinal approach, similar to the one used here, is essential in order to understand the dynamics of immunity and to determine whether responses indicate resistance or exposure across age classes (Woolhouse, 1992). It is possible that, in the case of co-infection with multiple parasite species, assessing responses to a specific parasite may not be indicative of overall resistance, and the type of immune response elicited may be more informative. For instance, T helper cell phenotype is reflected in cytokine production, measurement of which could be used to describe the functional balance of the immune system; for instance, high levels of the cytokines interleukin (IL)-4, IL-5, IL-9 and IL-13 are associated with responses against extracellular parasites such as helminths (Graham *et al*., 2007). Multivariate analysis of such measures could provide information about the direction of the immune response, as could groups of factors indicative of separate functional responses (Bradley & Jackson, 2008). These types of study are in their infancy in terms of their application to natural populations, and may be a fruitful avenue of future research in the attempts to better characterise the immunological basis of parasite resistance in wild animals.

Chapter 7

General Discussion

The aim of this thesis has been to investigate the associations between parasites and host life-history variation in the Soay sheep population of St Kilda. Throughout, heterogeneity in ageing and environmental conditions has been seen to exert an important influence, not only on parasites and life-history traits themselves, but also on the associations between the two. In chapter 2 I provided evidence for senescence in parasite resistance in late life, and showed that increased exposure to environmental stress over the life span accelerated the rate of senescence in females. In chapter 3 I investigated variation in parasite resistance across host life history in relation to aspects of maternal phenotype and early life performance, and showed that maternal age influenced offspring parasite resistance, and that some of the associations persisted into adult life. In chapter 4 I analysed age-specific variation in female reproductive traits, and showed that patterns of ageing and contributions of selective disappearance and terminal effects varied across traits. In chapter 5 I showed results consistent with positive selection on parasite resistance in lambs, but not in older sheep, and that selection was only present in relatively favourable environmental conditions. Finally, in chapter 6, I attempted to establish associations between a measure of parasite resistance and the strength of two antibody responses, showing that antibody responses were positively correlated and that there was a suggestion of a genetic component to this correlation. In this discussion, I consider firstly the associations between host age, parasite infection and variation in host life history, and then discuss the merits and drawbacks of the use of indirect measures of parasite resistance, with particular emphasis on the results presented in this thesis.

7.1 Ageing, parasites, and host life history

Here, I will discuss aspects of this thesis that have investigated the relationship between parasite infection and host senescence, and discuss the findings more widely in the context of the evolutionary ecology literature. I then discuss the development of resistance in early life and the importance of maternal effects. These results all highlight the importance of ageing in determining variation in host-parasite interactions, and indeed any phenotypic trait, in wild populations.

7.1.1 Patterns of senescence

A large number of studies have now that shown that senescence occurs in the two key demographic traits in natural populations, namely survival probability (e.g. Promislow, 1991; Ricklefs, 1998; Ricklefs & Scheuerlein, 2001; Wasser & Sherman, 2010) and reproductive performance (e.g. Nussey *et al*., 2009a; Vanpe *et al*., 2009; Zajitschek *et al*., 2009; Rebke *et al*., 2010; Sharp & Clutton-Brock, 2010). Interest has recently grown in measuring senescence in traits which may be indicators of physiological state or health. These include so-called 'biomarkers of ageing' (Johnson, 2006; Simm *et al*., 2008), such as telomere length (Monaghan, 2010), oxidative stress (Monaghan *et al*., 2009), and aspects of immune phenotype (De Martinis *et al*., 2005), as well as muscular condition (e.g. Hindle *et al*., 2009a; 2009b), hormone levels (e.g. Massot *et al*., 2011), and behavioural traits (e.g. Ridgel *et al*., 2003; MacNulty *et al*., 2009). However, longitudinal data on such traits are rare due to the difficulty of repeated capturing and sampling of individuals (Nussey *et al*., 2008). The available evidence suggests that declines in reproductive performance and survival with age are reflected in weakening immune function, since cross-sectional studies of birds have shown evidence for declines in measures of immunity in old age (e.g. Lozano & Lank, 2003;

Haussmann *et al*., 2005; Palacios *et al*., 2007). However, whether this is reflected in parasite burden, and what the implications are for host fitness, are poorly understood.

In chapter 2, I presented the first evidence for within-individual senescence in parasite resistance in a naturally-regulated population, and moreover, that the age-specific decline was accelerated by experience of poor environmental conditions. But how do these patterns reflect changes in other traits with age? The increase in FEC from middle age are reflected in changes in several aspects of reproductive performance (chapter 4), declines in a measure of annual fitness (Wilson *et al*., 2007) and reproductive performance in males (Robinson *et al*., 2006). However, these results are in contrast to others, which have found no evidence for individual age-specific changes in antibody responses in later life (Graham *et al*., 2010), no evidence for changes in a measure of oxidative damage across adult ages (Nussey *et al*., 2009b; a cross-sectional study), and recent work has shown that, independently of age, females show a decline in body mass only in the final year of life (Nussey *et al*., submitted). The results of chapter 4 also showed that the probability of twinning shows no significant decline at older ages. A recent study of wandering albatross (*Diomedea exulans*) investigated multiple aspects of reproductive performance, behaviour, and physiological measures of immunity, oxidative stress, and hormone levels, and found a decline in reproductive success that was mirrored in foraging behaviour but not any of the physiological indicators of ageing (Lecomte *et al*., 2010). Differences in the rates of actuarial and reproductive senescence were found in a study of Nazca boobies (*Sula granti*), which showed a small decline in survival probability in very late life, but a large decline in reproductive performance beginning in middle age (Anderson & Apanius, 2003). Finally, a study of great tits (*Parus major*) showed that the number of eggs produced, chicks hatched, and hatchlings fledged showed different age-specific change, with senescence being the earliest and most rapid for number of fledglings produced, and latest and slowest for number of eggs laid (Bouwhuis *et al*., 2009). Hence, investigations of ageing in Soy sheep and other wild vertebrates

emphasize that senescence is a complex process, and that though every trait is likely to show some kind of senescence, a marker of organism-wide senescence does not exist.

The lack of any age-specific decline in body weight suggests that this trait is under strong viability selection in Soay sheep (Nussey *et al*., submitted), and so females may invest more in their own condition than they do than in parasite resistance or in offspring. The declines seen in these two traits in later life could be indicative of age-specific declines in another trait, such as resource acquisition. It seems that here, there is no evidence for one of the common predictions of life-history theory, namely a trade-off between investment in immunity and reproduction (Folstad & Karter, 1992; Sheldon & Verhulst, 1996; Lochmilar & Deerenberg, 2000; Zuk & Stoehr, 2002), which has been vindicated in a number of theoretical and empirical studies (e.g. van Boven & Weissing, 2004; Hanssen *et al*., 2005; Mills *et al*., 2010). The results presented throughout this thesis suggest, if anything, positive covariance between life history traits, with heavy individuals showing higher fitness (chapters 4 & 5) and lower FEC (chapter 6), and lambs with lower FEC also showing higher fitness (chapter 5). Changes in resource acquisition with age could be estimated either through measures of tooth wear, which have been shown to be uncorrelated with age in red deer (Nussey *et al*., 2007b), or through estimates of forage intake rates (e.g. Jones *et al*., 2006), the latter of which has shown that, unsurprisingly, intake rate increases with body mass. Longitudinal studies of the former could be possible through dental moulds, but cross-sectional sampling of both could be informative.

7.1.2 Maternal effects and the development of immunity

Maternal care and resource provisioning can have substantial implications for early condition and survival of offspring, and these in turn may have knock-on effects on prospects in later

life (e.g. Mousseau & Fox, 1998; Marshall & Uller, 2007; Rasanen & Kruuk, 2007). The importance of the early environment, including maternal effects, on aspects of early offspring fitness has recently led to more investigations of such effects on offspring parasite resistance or immune function, which may contribute to or be associated with early survival prospects. The majority of studies on vertebrates, chiefly birds, have used a variety of assays for global measures of immune responses, as discussed in chapter 6, and have found that offspring immunity may be influenced by maternal exposure to antigens or parasites (Grindstaff *et al*., 2006; Reid *et al*. 2006) and maternal diet (Berthouly *et al*., 2008; Karell *et al*., 2008). Recently, the subject of maternal influences on lamb immunity has started to be addressed in Soay sheep. Preliminary analysis has shown that associations between maternal anti- *T. circumcincta* antibody (TcAb), measured in August, and lamb TcAb measured in the following April, vary across years of contrasting environmental conditions, and that lamb April antibody titre is positively associated with lamb weight in August (K. Morriss, MSc. Thesis, Princeton University). This suggests that high antibody levels could be positively correlated with maternal investment and good health, and is also reflected in the high heritability of TcAb reported in chapter 6. Early lamb antibody levels appear to be positively associated with lamb condition, but more analysis will be needed to determine whether this is associated with, for instance, neonatal survival. Such a result could indicate that maternal investment in transfer of immunity could be under positive selection via increases in lamb survival, since the results of chapter 5 show that lambs with low FEC have higher annual fitness (Hayward *et al*., in press). Whether this is subject to strong trade-offs with, for instance, ability to produce twins, maternal condition, or subsequent success or survival of mothers, remains to be seen, and such questions offer possible avenues of future research. We are only just beginning to scratch the surface.

Several studies have gone further and studied the downstream effects of maternal infection, antigen exposure, or immune priming of offspring, on aspects of offspring fitness. These

studies have the potential to improve our understanding of the extent to which maternal effects on offspring immunity are adaptive and have lasting positive effects on offspring fitness, or are simply passive indicators of maternal condition. For instance, Gallizzi *et al.* (2008a) showed that maternal exposure to fleas during egg-laying increased offspring body condition relative to offspring whose mothers were not infested with fleas; however, this effect was not apparent when brood size was artificially increased. It was suggested that maternal transfer of immunity had beneficial effects on early offspring fitness, in the light of evidence that ticks feed for less time on chicks whose mothers were exposed to fleas before laying (Gallizzi *et al.*, 2008b). However, the results of chapter 4 indicated that females with higher FEC had offspring that were less likely to survive to weaning (Table 4.5), suggesting a cost rather than a benefit of maternal infection in the Soay sheep and there is no evidence of a negative association between maternal and lamb FEC (Hayward *et al.*, 2010). Mothers with higher FEC have lower body weight (Coltman *et al.*, 2001a; Robinson *et al.*, 2009), and so it is likely that mothers with higher FEC are in lower condition and have fewer resources to invest in their offspring.

7.2 The interpretation of faecal egg counts

Throughout this thesis, and in many other studies on this population and others, an indirect measure of parasite burden, faecal egg count (FEC), has been used. As a non-invasive estimate of parasite burden, FEC are an excellent way in which to monitor variation in parasite resistance across seasons, years, and life history in a longitudinal manner. There are, however, several caveats to using FEC as an indicator of resistance. Below, I discuss previous investigations into the relationship between FEC and actual parasite burden in other wild populations, before discussing the implications of this relationship for the results of this thesis.

A major concern in interpreting FEC is the possibility of negative density-dependence in female worm fecundity. High parasite density may be associated with smaller worm size and lower fecundity due to competition for nutrients or space, with the result that at high worm burden egg production is reduced and FEC is lower than expected (Wilson *et al*., 2002). The obvious solution to this problem is to attempt to characterise the relationship between actual worm burdens and their indirect estimator by measuring the two simultaneously. The need to establish this relationship on a study-specific basis has been emphasized by the mixed findings of such attempts. Studies on a red grouse (*Lagopus lagopus scoticus*) population have shown that the relationship between worm number and FEC of the prevalent nematode *Trichostrongylus tenuis* is strong, linear, and does not vary across seasons (Moss *et al*., 1990; Seivwright *et al*., 2004), despite previous observations for seasonal heterogeneity in worm establishment (Shaw & Moss, 1989). However, in another game bird species, the ring-necked pheasant (*Phasianus colchicus*), there was evidence of strong density-dependence on fecundity of the prevalent nematode *Heterakis gallinarum*, with worm length beginning to decline at an intensity of 96 worms (Tompkins & Hudson, 1999). Added complexity may be introduced by considering that the relationship may vary seasonally and between parasite species, even within the same host. In a study of Svalbard reindeer (*Rangifer tarandus platyrhynchus*), the nematode *Ostertagia gruehneri* showed strong density-dependence across the year, while another species, *Marshallagia marshalli*, showed a complete absence of density-dependent fecundity (Irvine *et al*., 2001). It was also found that *O. gruehneri* fecundity was closely related to their size, but only during July, the period of most favourable transmission conditions (Irvine *et al*. 2001), suggesting that the influence of worm fecundity and number on FEC may vary across the year.

Evidence of density-dependent fecundity of strongyle nematodes has repeatedly been demonstrated in domesticated populations of sheep (e.g. Stear & Bishop, 1999; Bishop & Stear, 2000). Previous evidence for a linear association between FEC and worm burden in St

Kilda Soay sheep has been gained from post-mortem examination of individuals dying naturally during winters of high mortality and in extremely poor condition (Gulland, 1992; Grenfell *et al*., 1995; Wilson *et al*., 2004). However, it is possible that this relationship may not hold across all seasons, since climatic conditions and the nutritional state of sheep show intra-annual variation which may affect susceptibility to infection (Wilson *et al*., 2002). The relationship between FEC and adult worm burden, and the possibility that it may vary from the observed linear relationship, has important implications for the results presented in this thesis. Additionally, some of the results presented here may inform our understanding of the relationship in a population where the culling of healthy hosts is not possible.

Host factors are extremely important determinants of FEC, but it is of course also under the control of the worms themselves, and so if there are differences between hosts in the demography of their parasite fauna, this may change the relationship between worm number and FEC. For instance, the age-specific increase in FEC in adult sheep shown in chapter 2 could be a result of accumulating a greater proportion of older, larger, and more fecund worms, which would result in an elevated FEC for a given worm burden. However, there is evidence that nematode parasites themselves show declines in fecundity in old age (Train & Hansen, 1968), and strongyles have a maximum lifespan of around a year (Kassai, 1999), and so accumulation of more fecund worms by old sheep is unlikely to explain this pattern entirely. Since FEC is the product of worm number and size/fecundity, it may be related to the infection load in terms of the biomass of parasites. For instance, one sheep with a given FEC may harbour a few very large and fecund worms, while another may host many small and less fecund worms; if these infections have the same biomass, they may be expected to cause the same amount of damage. However, if the relationship between worm size and damage caused is non-linear, these two hypothetical infections may not be the same in terms of cost to the host in terms of physical damage or investment in immunity.

From an evolutionary ecological point of view, it is this effect of parasites on host fitness that is perhaps the most significant aspect of the relationship. FEC is assumed to be correlated with worm burden, which is expected to be correlated with a cost or damage, and so the value of FEC is as an indicator of the amount of stress the host is under due to parasite infection. It is therefore essential to establish the association between FEC and host fitness or condition, or valid conclusions cannot be made about the impact of a given FEC on the host, and nothing can be inferred about heterogeneity in immune phenotype. The results in chapter 5 show that the relationship between FEC and host fitness in Soay sheep is linear and negative in lambs, but that the two are uncorrelated in adults (Hayward *et al*., in press). The linear relationship in lambs could arise because at four months of age immunity is relatively low compared to adults, and so may not regulate worm fecundity to the same extent (Smith *et al*., 1985). The lack of phenotypic selection on FEC in adults could in part explain the increase in FEC in late life shown in chapter 2, and it is particularly informative to compare this to the corresponding observations with regard to body weight. As females may command fewer resources in old age, there is strong selection to maintain body weight, which does not decline until the final year of life (Nussey *et al*., submitted), while there is only weak selection to invest in reducing FEC, which as a result increases in later life (Hayward *et al*., 2009). The results of chapter 3 also suggest that weak selection on FEC in adult females may have implications for lamb fitness, since older females have lambs with high FEC which therefore have lower subsequent fitness.

A final caveat of FEC is that it does not reflect levels of larval stages or immature adults. This could be especially relevant in the attempts to investigate associations between FEC and antibody titres in chapter 6, since strong IgG responses are also mounted against fourth stage larvae which do not contribute to FEC (Stear *et al*., 1995). High antibody titres could therefore be reflective of a recent ingestion of larvae rather than associated with adult worm

burden, and therefore it may not be surprising that anti-*T. circumcincta* antibody (which is likely to be cross reactive to antigens of larval stages) is not strongly associated with FEC.

Thus, although FEC and other indirect measures are extremely useful indicators of parasite resistance, they can only take us so far when used in isolation. In the next section, I discuss potential future avenues of research which may enable us to better understand the associations between hosts and parasites in natural populations.

7.3 Future prospects

The above discussion highlights some of the important findings across fields of research relevant to the major themes of this thesis, but also shows how much there is still to be done. A key subject to address was discussed extensively in chapter 6, namely that choosing relevant measures of parasite resistance and immunity will be crucial in ecological studies of host-parasite systems. In terms of research on natural host-parasite associations with respect to ageing, a key development will be the availability of longitudinal data on a combination of immune responses, parasite infection intensities, and fitness, since only longitudinal data can reveal individual-level patterns of ageing (Nussey *et al*., 2008). With pedigree data, they can also investigate the genetic architecture of phenotypic traits, and their changes across ages (e.g. Charmantier *et al*. 2006b; Brommer *et al*., 2007; Wilson *et al*. 2007; Wilson *et al*., 2008). As indicated above, study populations on which such data could be collected in a longitudinal manner are relatively rare, especially in terms of mammals.

A recent review has highlighted the need for such data, and has proposed that modification of existing laboratory techniques for closely-related wild populations could be a fruitful avenue of future research (Pedersen and Babayan, 2011). For this reason, and because of

ease of recapture, populations of wild rodents were suggested as having the potential to give valuable insight into the relationships between parasites, immunity, age and environmental heterogeneity. For instance, the wild rodent populations of Kielder Forest in northern England have already been the subject of extensive epidemiological and parasitological research, including the impact of parasites on survival and health (Telfer *et al*., 2002; Beldomenico *et al*., 2008a; 2008b), the seasonality of infections (Begon *et al*., 2009), and the dynamics of co-infection (Telfer *et al*., 2007; 2008; 2010). All of these studies have been longitudinal, but an advantage of wild rodents is that they can be used for invasive sampling, such as in a recent study on the same population which measured cytokine expression in splenocyte cultures (Jackson *et al*., 2011).

The Soay sheep study population is unique in being a longitudinal mammal study with extensive life-history data as well as a cache of stored blood samples from around twenty years. An advantage of the sheep over rodents is that being a relatively large mammal, large quantities of blood can be collected and aliquots taken for numerous simultaneous assays. However, a limitation is the difficulty of sampling individuals repeatedly over short periods; for instance in order to monitor the development of immunity in lambs in relation to their condition over their first few weeks of life. Individuals are sampled at most once a year in August, which puts limitations on studying immune responses in relation to key life-history events; for instance, during the lambing season both females and males experience higher FEC (Wilson *et al*., 2004) but it is unknown how this is related to immune function. It may be possible to carry out small-scale intensive sampling in a cross-sectional manner to answer some of these questions. The analyses presented in this thesis have been based on observational data, and it may be that experimental studies could shed further light on some of the results herein. For instance, if sections of the study area could be cordoned off, individuals of different ages could be treated with anthelmintic and then re-infected with strongyle larvae, either standardized by body weight or of varying doses, perhaps across

varying patch quality. Close monitoring of faecal egg count, body weight, and blood sampling could reveal the dynamics of infection across, for instance, sexes or age categories. The first two weeks could be particularly interesting, because this is the period before eggs appear in faeces; once eggs did start to appear, an understanding could be gained of how they relate to aspects of immunity and to the initial dose. For instance, it might be predicted that younger animals, males, and very old animals would produce higher FEC from a given dose due to a less effective response; comparisons could also be made with domestic flocks, as has been done with wild and laboratory mice, *Mus musculus* (Abolins *et al*., 2011). Even without experimental studies, the quality of data from the Soay sheep population still carries the prospect of being able to answer some of the questions related to ageing, parasites and immunity that are yet to be investigated in natural populations.

7.4 Final thoughts

The original research in this thesis illustrates the importance of longitudinal study of host-parasite interactions in heterogeneous environmental conditions. Only longitudinal studies can reveal the true patterns of ageing, selection, and covariance between phenotypic traits. Study systems such as the Soay sheep of St Kilda, which combine repeated capture of known individuals, as well as knowledge of prevailing environmental conditions, have great potential to advance our understanding of interactions between host fitness, immunity, and parasites in natural populations. These findings, and future work, should be of great relevance to a multitude of research themes, including life-history theory, the evolution of ageing, and immunology, with implications for disease management and conservation.

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Appendix A

Chapter 4 supplementary results

Presented below are final models from analysis testing for significant late-life declines in reproductive performance (section 4.3.3). These results are described in section 4.4.2.

Trait	Variable	Estimate	S.E.	d.f.	χ^2	p
a) Annual Fecundity						
	Fixed effects					
	Intercept	-5.9068	1.5016		-3.934	<0.001
	ALR	2.3090	0.3472	1	6.650	<0.001
	ALR²	-0.0895	0.0197	1	-4.539	<0.001
	Age	-0.7443	0.0620	1	-12.005	<0.001
	Variance components					
	Year	0.5173	0.0201			
b) Probability of twinning						
	Fixed effects					
	Intercept	-1.6859	0.2028	1	-8.313	<0.001
	Variance components					
	ID	1.7193	0.0792			

Table A1: Final models of analysis of a) annual fecundity and b) probability of twinning in females of post-peak age (6 and 8 respectively). Results from GLMMs with binomial errors described in section 4.3.3.2. Sample sizes are shown in Table 4.7.

Trait	Variable	Estimate	S.E.	d.f.	Test stat.	p
a) Offspring birth weight						
	Fixed effects					
	Intercept	-2.5082	0.7901			
	Lamb Sex					
	Female	0.0000		NA	10.46	0.001
	Male	0.2083	0.0640			
	Birth Date	0.0372	0.0062	NA	35.38	<0.001
	PPD	-0.0021	0.0004	NA	17.53	<0.001
	NAO	-0.1101	0.0462	NA	5.17	0.023
	ALR	0.0761	0.0406	NA	3.48	0.062
	Age	-0.1399	0.0293	NA	22.29	<0.001
	Variance components					
	ID	0.3142	0.0218			
	YEAR	0.0304	0.0068			
	RESIDUAL	0.5255	0.0282			
b) Offspring growth rate						
	Fixed effects					
	Intercept	0.0514	0.0155			
	Offspring Weight	0.0105	0.0010	NA	94.38	<0.001
	Lamb Sex					
	Female	0.0000		NA	76.73	<0.001
	Male	0.0118	0.0013			
	Birth Date	0.0003	0.0001	NA	8.01	0.005
	PPD	0.0000	0.0000	NA	8.31	0.004
	ALR	0.0020	0.0006	NA	12.47	<0.001
	Age	-0.0033	0.0005	NA	48.27	<0.001
	Variance components					
	ID	0.0001	0.0004			
	YEAR	0.0000	0.0002			
	RESIDUAL	0.0002	0.0006			
c) Offspring survival						
	Fixed effects					
	Intercept	5.6362	1.0085		5.59	<0.001
	Lamb Sex					
	Female	0.0000		1	-2.90	0.004
	Male	-0.7603	0.2622			
	Offspring Weight	1.3213	0.1701	1	7.77	<0.001
	ALR	0.0239	0.1183	1	0.20	0.840
	Age	-0.3590	0.1018	1	-3.53	<0.001
	Variance components					
	ID	0.9631	0.0322			
	YEAR	0.6443	0.0264			

Table A2: Final models of analysis of maternal effects-related traits from females of post-peak age: a) offspring birth weight OWT; b) offspring growth rate OGR; c) offspring survival OS. Results from LMMs with normal errors for OWT and OS, and from GLMM with binomial errors for OS; both are described in section 4.3.3.2. Sample sizes are shown in Table 4.7.

Appendix B

Publications related to this thesis

Graham, A.L., Hayward, A.D., Watt, K.A., Pilkington, J.G., Pemberton, J.M. & Nussey, D.H. (2010) Fitness correlates of heritable variation in antibody responsiveness in a wild mammal. *Science* **330**, 662-665.

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Hayward, A.D., Pilkington, J.G., Pemberton, J.M. & Kruuk, L.E.B. (2010) Maternal effects and early-life performance are associated with parasite resistance across life in free-living Soay sheep. *Parasitology* **137**, 1261-1273.

Hayward, A.D., Wilson, A.J., Pilkington, J.G., Clutton-Brock, T.H., Pemberton, J.M. & Kruuk, L.E.B. (2011) Natural selection on a measure of parasite resistance varies across ages and environmental conditions in a wild mammal. *Journal of Evolutionary Biology*.